Atty Docket No. 056100-5027

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

LEE

Serial No.: 08/966,233

Filed: November 7, 1997

Title: GDF-1 GENE

Group Art Unit: 1631

Examiner: M.P. Allen

#66
Plunkett
5/22/03

DATE: May 14, 2003

APPELLANT'S APPEAL BRIEF

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

Introduction

The invention on appeal encompasses isolated nucleic acids encoding a mammalian GDF-1 protein.

Real Party in Interest

The real party in interest is the Carnegie Institution of Washington by virtue of assignment from the inventor, Dr. Se-Jin Lee, recorded at Reel 5582, Frame 0797 on January 16, 1991. An exclusive license is currently held by Curis, Inc.

Related Appeals and Interferences

There are no related interferences known to the appellant, his legal representatives or assignee which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal. An appeal is being concurrently filed in related divisional application Serial No. 08/971,338.

05/16/2003 HAHNED1 00000033 500310 08966233
01 FC:2402 160.00 CH

Status of Claims

Claims 1-21 were presented with the application as filed and claims 22-42 were added by amendment. Of these claims, claims 2, 4-10, 16-21 and 23 have been canceled. Claims 3, 11-15, 22 and 24-42 are pending and at issue in this appeal, and are set out in an Appendix to this brief (Exhibit A).

Status of Amendments

The appellant submitted a response dated July 12, 2002 with arguments in response to the non-final Office Action dated February 11, 2002. This response was entered and considered in the Final Office Action dated October 21, 2002. The appellant filed a Notice of Appeal on January 21, 2003. An amendment after final was submitted on March 21, 2003, which appellant believed narrowed the issues for appeal. The Examiner refused entry of this amendment.

Summary of the Invention

In concise form, the invention of the pending claims is directed to the isolation of nucleotide sequences encoding the GDF-1 gene, recombinant molecules and vectors comprising those sequences, cells transformed with such recombinant molecules, and methods of producing recombinant GDF-1 protein.

The GDF-1 gene was isolated by virtue of its homology to the transforming growth factor beta (TGF-β) superfamily. A growing number of polypeptide factors playing critical roles in regulating differentiation processes during embryogenesis have been found to be structurally homologous to the TGF-β superfamily (see the Background of the Invention in the specification). Potential uses for GDF-1 as a therapeutic and diagnostic tool are suggested based on the known biological activities of other members of this superfamily (pages 12-14 of the specification and particularly page 13, lines 16-18).

The sequence for murine GDF-1 was originally isolated from a cDNA library prepared from day 8.5 embryos, which was screened using oligonucleotides selected on the basis of amino acid sequences of conserved regions among members of the TGF-β superfamily (see Example 1 of the specification, page 18). The sequence of one 1.4 kb GDF-1 clone obtained is shown in Figure 2, and contains a single long open reading frame encoding a protein of 357 amino acids,

beginning with an initiating ATG codon at nucleotide 217. Two other clones from the library were sequenced, and are believed to represent allelic variations within the gene (see page 19, lines 17-29).

A comparison of the C-terminal region of the GDF-1 protein with other members of the TGF-β superfamily is shown in Figure 3a. The GDF-1 sequence contains all of the invariant amino acids present in the other members, including the seven cysteine residues with their characteristic spacing, and a pair of arginine residues at positions 236-237. The level of homology observed between GDF-1 and the other members of the TGF-β superfamily (26-52%) was high enough to assign GDF-1 to this family but not so high so as to suggest that GDF-1 was a homologue of a previously-identified TGF-β family member (see page 20, lines 7-30).

To determine whether GDF-1 is a single copy gene, Southern hybridization to mouse genomic DNA was carried out using the GDF-1 cDNA as a probe (see Example 3, page 22). A predominant band was identified in three different digests of mouse genomic DNA. Further, the GDF-1 probe also detected a single prominent band in both human and hamster genomic DNA, even at high stringency, suggesting that GDF-1 is highly conserved across species (page 22, lines 24-32).

When the inventors examined GDF-1 expression in embryos, a 1.4 kb transcript was observed in embryos at day 8.5 and 9.5, but not in later stage embryos. A second mRNA species, 3.0 kb in length, appeared at day 9.5 and persisted throughout embryogenesis (page 23, lines 9-13). Examination of adult animals showed that the 3.0 kb transcript was also present almost exclusively in the central nervous system of adult animals (pages 23-24). The 3.0 kb transcript was cloned from a murine adult mouse brain cDNA library, and was shown to contain both the 1.4 kb segment comprising the GDF-1 open reading frame, and a 1527 base pair region upstream (Example 7, page 28). This upstream region was found to contain a second long open reading frame beginning with an initiating ATG codon at position 74, extending 350 codons, and terminating 404 base pairs upstrem of the GDF-1 open reading frame. This second open reading frame was designated UOG-1 (upstream of GDF-1) (see paragraph bridging pages 29-30). A search of the NBRF and GenBank sequence databases revealed no known sequences with

significant homology to UOG-1 (page 30, lines 5-10). Figure 11A shows the complete sequence of a mouse 2.7 kb clone containing both the UOG-1 and GDF-1 open reading frames.

Human GDF-1 cDNAs were also isolated from adult cerebellum and fetal brain cDNA libraries using the murine GDF-1 cDNA as a probe (Example 8, page 30). Figure 11B shows the sequence of a 2510 base pair human cDNA containing the open reading frame for human GDF-1 beginning at nucleotide 1347 and an upstream open reading frame that is 81% identical on the amino acid level to murine UOG-1. The murine and human sequences are 87% identical in the region beginning with the first conserved cysteine and extending through the C-terminus, and 69% identical through the entire length of the proteins. Southern hybridization to genomic DNA was performed to verify that the human GDF-1 gene is indeed the homolog of the mouse GDF-1 gene (see paragraph bridging pages 31-32).

Example 6, beginning on page 25 of the specification, provides a protocol for cloning GDF-1 into an expression vector and expressing GDF-1 in a host cell to produce recombinant GDF-1 protein.

Thus, the pending claims are fully supported by the appellant's original disclosure.

The Issues

(1) Utility and Enablement

The Examiner has finally rejected claims 3, 11-15, 22, and 24-42 under 35 U.S.C. §101 because the invention is allegedly not supported by a specific, substantial and credible utility or by a well established utility. The Examiner has made a corresponding rejection of these claims under 35 U.S.C. §112, first paragraph for lack of enablement, alleging that since the claimed invention is not supported by a utility, one of skill in the art would not know how to use the claimed invention. Since the Examiner has supported these rejections with a single argument, the rejections are considered in this brief as a single issue. On this basis, the issue can be stated as follows:

> Are the appellant's claimed nucleic acids lacking in either a specific, substantial and credible utility or a well-established utility such that one of skill in the art would not know how to use the claimed invention?

The appellant submits that the claimed nucleic acids are supported by a specific, substantial and credible utility such that the skilled artisan would know how to use the claimed invention for reasons detailed herein.

(2) Written Description

The Examiner has finally rejected claims 3, 11-15, 22, and 24-42 under 35 U.S.C. §112, first paragraph, for allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the invention. The Examiner's general basis for the rejection is that appellant has not provided defining structural or functional features for GDF-1 and therefore the appellant has not described the invention as broadly as claimed. In particular, the Examiner asserts that some of the claims, including the hybridization-based claims, read on genomic sequences, and that the specification does not describe the structure of genomic sequences. The Examiner further asserts that some claims read on sequences outside of the disclosed open reading frame that have not been disclosed and are therefore not described. This rejection therefore raises several issues that pertain to different groupings of claims as described in more detail below. The issues can be stated as follows:

> Has the appellant provided defining structural or functional features of a GDF-1 gene so as to provide adequate description for the claimed genus?

and more specifically for some claims,

> Does the specification reasonably convey to one skilled in the art that the inventors had possession of sequences that hybridize to the disclosed coding sequences under the claimed conditions at the time the application was filed?

and more specifically for other claims,

> Does the specification reasonably convey that the inventors were in possession of nucleic acid sequences comprising the disclosed open reading frame sequences at the time the application was filed?

For the reasons detailed below, the appellant submits that the specification provides defining structural features of a GDF-1 gene so as to provide support for the claimed genera, and

furthermore, that the skilled artisan would immediately recognize that the appellant was in possession of the claimed genera, including sequences that hybridize to the disclosed sequences and sequences comprising the disclosed sequences, at the time the application was filed.

(3) New Matter

The Examiner has finally rejected claims 39-42 under 35 U.S.C. §112, first paragraph, for allegedly containing new matter. Specifically, the Examiner asserts that the appellant has not pointed to a page and line number showing support for these claims. On this basis, the issue can be stated as follows:

Do claims 39-42 contain new matter so as to be unpatentable under 35 U.S.C. §112, first paragraph, for lack of written description?

For the reasons detailed below, the appellant submits that claims 39-42 do not contain new matter, and that appellant has provided page and line number support for these claims.

Grouping of Claims

Claims 3, 11-15, 22 and 24-42 may be considered together with regard to the utility and enablement (how to use) arguments.

The claims have been separated into three distinct groups for the written description arguments: (1) claims 22, 3 and 11-15, (2) claims 31-34 and 39-42, and (3) claims 24-30 and 35-38. These groups should each be considered exclusively.

Claims 39-42 may be considered together with regard to the new matter rejection.

Arguments

(1) Utility and Enablement

The Examiner has finally rejected claims 3, 11-15, 22 and 24-42 under 35 U.S.C. §101 because the invention is allegedly not supported by a specific, substantial and credible utility or by a well established utility. The Examiner has made a corresponding rejection of these claims under 35 U.S.C. §112, first paragraph for lack of enablement, alleging that since the claimed

invention is not supported by a utility, one of skill in the art would not know how to use the claimed invention. The appellant respectfully requests that the Board reverse this rejection.

As described in the first sentence of the specification, the invention relates to DNA segments encoding proteins of the transforming growth factor beta superfamily. As described in the Background Information section at page 1, lines 15-19, a growing number of polypeptide factors playing critical roles in regulating differentiation processes during embryogenesis have been found to be structurally homologous to transforming growth factor β (TGF-β). Further, as reiterated at page 2, lines 23-28 of the specification, the invention relates to a new member of the TGF-β superfamily, which, like other members of this superfamily, is predicted to play an important role in mediating developmental decisions related to cell differentiation.

As further described on page 12 of the specification, beginning at line 10:

> The structural homology between GDF-1 and the known members of the TGF-β superfamily and the pattern of expression of GDF-1 during embryogenesis indicate that GDF-1 is a new member of this family of growth and differentiation factors. Based on the known properties of the other members of this superfamily, GDF-1 can be expected to possess biological properties of diagnostic and/or therapeutic benefit in a clinical setting.

The specification goes on to provide several potential uses for GDF-1 based on the observed homology to the TGF-β superfamily, including diagnostic use as an indicator for the presence of developmental anomalies in prenatal screens for potential birth defects (page 12, lines 32-35). This proposed use is directly related to the predicted role of GDF-1 in embryogenesis, and is supported by the fact that other members of the TGF-β superfamily known at the time had each been shown to play a pivotal role in embryonic processes. See Akhurst *et al.*, 1990, Prog. Growth Factor Res. 2(3): 153-68 (abstract) (attached as Exhibit B).

In the Office Action dated February 11, 2002, the Examiner dismissed the noted homology of GDF-1 proteins to the TGF-β superfamily as failing to provide the requisite utility, because the activities of the members of the TGF-β superfamily "vary quite widely," and "some TGF superfamily members have diverse activities in embryonic development and some have no

role in development" (Office Action, pages 4 and 5, respectively). The Examiner provided no documentary evidence to support this assertion.

Appellant respectfully notes that the Akhurst reference was published in 1990, which is the year that the earliest priority application to the present application was filed. Therefore, the Akhurst reference is an appropriate measure of what was known in the art relating to transforming growth factors at the time the application was filed. According to the Akhurst abstract, "each" of the TGF-β superfamily members identified at that time plays "a pivotal role" in embryonic processes. There is no suggestion that other TGF-β family members known at that time played a role that did not relate to development, and the Examiner has provided no evidence to suggest that TGF-β members known at that time were thought to "have no role in development."

Thus, at the time the present application was filed, the TGF-β superfamily had been characterized as "a large superfamily of related proteins, each of which plays a pivotal role in embryonic processes" (Akhurst). It is Appellants' understanding upon a reading of the utility guidelines (FR, Vol. 66, No. 4, January 5, 2001) (Exhibit C) that it is perfectly acceptable to assert a specific, substantial and credible utility on the basis of "homology to existing nucleic acids or proteins having an accepted utility." According to the FR Notice, a rigorous correlation is not necessary; only a "reasonable" correlation (see the FR Notice, page 1096, middle column continuing into right-hand column). As stated therein, "When a class of proteins is defined such that the members share a specific, substantial, and credible utility, the reasonable assignment of a new protein to the class of sufficiently conserved proteins would impute the same specific, substantial, and credible utility to the assigned protein" (with emphasis). Id.

The Examiner has not disputed the reasonable assignment of GDF-1 to the TGF-β superfamily. As acknowledged by the Examiner, GDF-1 proteins are 26-52% similar to TGF-β family members on the amino acid level (Office Action dated February 11, 2002, page 4). Moreover, according to the specification at the paragraph bridging pages 19-20, GDF-1 contains all of the invariant amino acids present in the C-terminal 122 amino acids of other TGF-β superfamily members, including the seven characteristic cysteine residues as well as many of the other most highly conserved amino acids. For instance, like the other family members, the

C-terminal portion of the predicted GDF-1 polypeptide is preceded by a pair of arginine residues at positions 236-37. Thus, GDF-1 contains sufficient homology to be reasonably assigned to the TGF-β superfamily.

According to the new utility guidelines, "the asserted utility must be accepted by the examiner unless the Office has sufficient or sound reasoning to rebut such an assertion." Id. (with emphasis). The Examiner rejects the asserted utility on the basis that the members of the TGF-β family exhibit diverse activities, and that some members have roles not related to embryonic development. However, the Examiner provides no evidence that those of skill in the art at the time the invention was made would have believed that members of the TGF-β super family exhibit such diverse activities as to preclude prediction of function based on this family assignment. In contrast, according to the Akhurst abstract, there had been five type beta transforming growth factors (TGF-βs) identified at the priority date of the invention, each of which was found to play "a pivotal role in embryonic processes."

Thus, at the time the invention was made in 1990, one of skill in the art would have reasonably predicted that a member assigned to the TGF-β superfamily would play a role in embryonic development, and in the growth and differentiation of tissues, given that at least five TGF-β members identified at that time were shown to play a "pivotal role" in embryonic development. Indeed, as mentioned above, according to the instant specification at page 1, "a growing number of polypeptide factors playing critical roles in regulating differentiation processes during embryogenesis [had] been found to be structurally homologous to transforming growth factor B." On that basis, and in view of the homology of GDF-1 to TGF-β, the appellant predicted that the GDF-1 protein was likely to play an important role in mediating developmental decisions related to cell differentiation (see page 2, lines 25-29). Moreover, it was perfectly reasonable on the basis of that prediction and the homology demonstrated according to the rules promulgated by the Office for Applicants to assert that the claimed protein would find utility in prenatal screens to detect developmental abnormalities, as disclosed on pages 12-13 of the specification.

The reasonable assignment of utilities based on predicted role in embryonic development is further confirmed by recent experiments that show that, in fact, the appellant's predictions

were correct. As the present inventor and others have shown in a recently published paper (Rankin *et al.*, 2000, Regulation of left-right growth patterning in mice of growth/differentiation factor-1, Nature Genetics 24: 262-66) (Exhibit D), GDF-l plays a pivotal role in embryogenesis. A knockout mouse was generated in order to examine the biological function of GDF-1, which exhibited a spectrum of defects related to left-right axis formation in embryos, including misplacement of internal organs (Fig. 2), developmental defects in organs and cardiac abnormalities (Fig. 3). The authors concluded that these findings indicate that GDF-1 is essential for proper establishment of the left-right axis in mice, and is required for the expression of many genes expressed downstream from GDF-l during development.

The Examiner dismisses the Rankin reference because it was published well after the filing date of the invention, and because knock-out mice were not routinely produced at the time of the invention. It is appellant's understanding, however, that it is acceptable to submit evidence gathered after the filing date to prove the adequacy and accuracy of the specification disclosure. The Rankin reference was submitted to demonstrate that the GDF-1 protein has the utilities that were predicted in the specification, and is suitable evidence for that purpose even though it was published after the filing date of the present application.

Thus, results with the GDF-1 knockout mouse prove that GDF- 1 is required for the proper development and positioning of organs during embryogenesis. This is consistent with the function of GDF-l predicted in the specification (page 2, lines 25-29), and the results in the specification showing the expression of GDF-1 during embryogenesis (see Example 4 and Fig. 6). These results also confirm that the asserted utility of GDF-1 in prenatal screens for abnormal development is a reasonable utility for the disclosed nucleic acid, given that it has now been confirmed that aberrant expression of GDF-1 has significant and substantial effects on embryonic development.

In the Office Action dated October 21, 2002, the Examiner argues that one of skill in the art would not interpret the gene expression results in Example 4 and Figure 6 as a prediction that the biological role of GDF-1 would be the regulation of left-right patterning or axis formation in mice (page 3). However, the appellant has not asserted that the specification teaches that GDF-1 regulates left-right patterning or axis formation in mice. Rather, appellant predicted in the

specification that GDF-1 plays a role in embryonic development, and the showing in Rankin *et al.* that GDF-1 specifically regulates left-right patterning and axis formation during embryonic development is proof that appellant's prediction was correct.

The Examiner further argues that the information in the specification does not suggest or predict a prenatal condition that GDF-1 could be used to screen for (Office Action dated October 21, 2002, page 3). This is not correct, however, because the specification discloses at the paragraph bridging pages 12-13 that abnormal levels of GDF-1 could be associated with developmental anomalies or structural defects in the developing fetus.

Thus, at the time the application was filed, the TGF-β superfamily was known to comprise proteins involved in embryonic development, a function that appellant predicted that GDF-1 would share. Further, appellant has demonstrated according to published evidence (Rankin *et al.*) that GDF-1 does possess the predicted function, thereby supporting the disclosed utilities, *i.e.*, use in prenatal screening for developmental defects. And as noted above, according to the utility examination guidelines, it is perfectly acceptable to predict a specific, substantial and credible utility on the basis of homology to an established family of nucleic acids or proteins having an accepted utility. The claimed nucleic acids are therefore supported by the specific, substantial and credible utility as a marker for the presence of embryonic developmental and structural defects, and the skilled artisan would therefore know how to use the claimed invention.

The appellant has offered evidence of other utilities disclosed by the specification over the course of prosecution that have also been dismissed by the Examiner. For instance, as described in the specification at the top of page 14, "If GDF-1 possesses a similar activity [as the TGF-β family member, activin], as is indicated by its specific expression in the central nervous system, GDF-1 will likely prove useful *in vitro* for maintaining neuronal cultures." To demonstrate that GDF-1 possesses the predicted function, the appellant submitted a declaration pursuant to 37 CFR §1.132 by Dr. Ted Ebendal (Exhibit E) on April 24, 1998, describing experiments showing that GDF-1 has a biological activity on neurons in culture similar to other TGF-β family members.

The Examiner indicated that the Ebendal declaration was not sufficient because the information presented in the declaration was not known at the time the application was filed

(Office Action, February 22, 1999, page 7). Again, it is the appellant's understanding that it is perfectly acceptable to submit evidence gathered after the filing date of the application to demonstrate that the utilities predicted in the specification were correct. The Ebendal declaration is therefore suitable evidence supporting the predicted activity of GDF-1 as a cell survival molecule in the *in vitro* culturing of neurons and is acceptable evidence rebutting the rejection.

Further, as described in the Appeal Brief submitted July 3, 2000, DNA segments containing GDF-1 are also useful as cell lineage markers. Indeed, as stated in the specification at page 12, lines 20-23, "One potential use for GDF-1 as a diagnostic tool is as a specific marker for the presence of tumors arising from cell types that normally express GDF-1." Figure 7 of the specification shows that GDF-1 is expressed almost exclusively in the brain. Thus, a GDF-1 nucleic acid may be used to determine for instance whether a brain tumor is a primary tumor or a metastasis from a tissue that does not express GDF-1. Such a determination has diagnostic and therapeutic consequences for cancer treatment, and is fully enabled by the showing of the tissue expression profile of GDF-1 in the original specification.

In view of all the remarks above, the appellant submits that the claimed invention is supported by at least one specific, substantial and credible utility, therefore, the skilled artisan would know how to use the claimed invention.

(2) Written Description

Claims 22, 3 and 11-15

The Examiner has finally rejected claims 22, 3 and 11-15 under 35 U.S.C. §112, first paragraph, for allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the invention. The Examiner's basis for the rejection is that the appellant has not provided defining structural or functional features for GDF-1 and therefore the appellant has not described the invention as broadly as claimed.

At the outset, the appellant respectfully notes that the nucleic acid sequences encompassed by claims 22, 3 and 11-15 are defined according to the specific GDF-1 amino acid sequences disclosed in the specification. Therefore, the specification adequately discloses the

claimed invention. For instance, independent claim 22 is directed to an isolated DNA segment encoding a mammalian GDF-1 protein having the amino acid sequence defined in an open reading frame of Figure 2 or Figure 11A or Figure 11B. Thus, all DNA segments encompassed by this claim must encode one of the specific GDF-1 protein sequences disclosed in the specification.

The specification discloses nucleic acid sequences encoding GDF-1 proteins from both human and mouse, and also provides the sequences of several allelic variants that were separately cloned (see Examples 1, 7 and 8 in the specification, and particularly page 19, lines 17-29, and the paragraph bridging pages 28-29). In particular, one of the allelic variants identified contained a nucleotide variation that did not alter the amino acid sequence (page 19, lines 23-25). Nevertheless, even if the specification had not identified any allelic variants encoding the same GDF-1 amino acid sequence, one of skill in the art would clearly see that the degeneracy of the triplet code of DNA allows for nucleotide substitutions in the coding sequence that do not change the corresponding amino acid sequence.

Given that claims 22, 3 and 11-15 are limited to isolated DNA segments encoding the specific mammalian GDF-1 amino acid sequences that are disclosed in the specification, and given that those of skill in the art are well aware that the nature of the triplet code allows for degeneracy in protein coding sequences, appellant respectfully submits that the skilled artisan would immediately recognize that the appellant was in possession of the genus recited in claims 22, 3 and 11-15 at the time the application was filed.

Claims 31-34 and 39-42

The Examiner has finally rejected claims 31-34 and 39-42 under 35 U.S.C. §112, first paragraph, for allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the invention. The Examiner's basis for the rejection is that the appellant has not provided defining structural or functional features for GDF-1 and therefore the appellant has not described the invention as broadly as claimed. In particular, the

Examiner asserts that these claims also read on genomic sequences, and that the specification does not describe the structure of genomic sequences.

At the outset, the appellant respectfully notes that claims 31-34 and 39-42 are not directed specifically to genomic sequences, but rather to sequences that hybridize to the specific nucleic acid sequences disclosed in the specification under the specific hybridization conditions disclosed. Thus, the appellant respectfully submits that the pending claims, worded in terms of hybridization conditions that are clearly recited in the specification are supported by the specification.

Indeed, according to the Synopsis of Application of Written Description Guidelines available on the U.S. Patent & Trademark Office's internet site (attached as Exhibit F), it is perfectly acceptable to define the claimed genetic sequences in terms of the hybridization conditions disclosed in the specification. For instance, in Example 9 of the Training Examples provided in the Synopsis beginning on page 35,[1] an example is provided where the specification discloses a single cDNA (SEQ ID NO: 1) encoding a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity. The specification discloses that the complement of SEQ ID NO: 1 was used under highly stringent hybridization conditions for the isolation of nucleic acids that encode proteins that bind to dopamine receptor. The hybridizing nucleic acids were not sequenced. The exemplary claim is directed to an isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 1, wherein the nucleic acid encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity.

Example 9 of the Synopsis acknowledges that the claim is drawn to a genus of nucleic acids all of which must hybridize with SEQ ID NO: 1 and must encode a protein with the same activity. The example also acknowledges that, in this instance, there is only a single species disclosed that is within the scope of the genus. Nevertheless, the example also notes that the art indicates that hybridization techniques using a known DNA probe under highly stringent conditions were conventional in the art at the time of filing (see Analysis beginning on page 36).

[1] The Table of Contents of the Synopsis erroneously indicates that Example 9 begins on page 28.

The example further notes that a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claim because the highly stringent conditions set forth in the claim yield structurally similar DNAs. Thus, "a representative number of species is disclosed, since highly stringent hybridization conditions in combination with the coding function of the DNA and the level of skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention" (see sentence bridging pages 36-37).

Like the claim at issue in Example 9 of the PTO's Written Description Guidelines Synopsis discussed above, appealed claims 31 and 39 are directed to a genus of isolated DNAs encoding the described protein (GDF-1) that hybridize under specific hybridization conditions to the nucleotide sequence for GDF-1 that is specifically disclosed in the specification. However, rather than the single species disclosed in the specification of Example 9, the instant specification discloses two species with the genus (human and mouse cDNA sequences), and analyses the homology between the two. Further, as in the Synopsis Example 9, hybridization techniques using a known DNA probe under highly stringent conditions were conventional in the art at the time of filing, and a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claim because the highly stringent conditions set forth in the claim yield structurally similar DNAs. Therefore, based on the teachings of the Synopsis of Application of Written Description Guidelines, appealed claims 31 and 39 (and the claims dependent thereon) should be found to have written description support in the specification.

Example 9 in the Synopsis does not address whether the genus encompassed by the exemplary claim reads on genomic sequences that have not been disclosed, as asserted by the Examiner. In any case, to the extent that the appealed claims do read on genomic GDF-1 sequences, the instant specification does show that a probe generated from the full length murine open reading frame of GDF-1 hybridizes specifically to the human gene in Southern hybridization (see Fig. 14 legend at page 9, and the relevant discussion at pages 31-32). As also shown in Figure 5, even at high stringency, a murine GDF-1 probe identified a single prominent band in both human and hamster genomic DNA. The genomic sequences identified by these

hybridization experiments could be readily cloned and sequenced using techniques that were well known at the time the application was filed.

The Examiner has not explained why this disclosure is not sufficient, except to note that additional bands were detected in the Southern blot experiments in Example 5, even under high stringency hybridization conditions (see Office Action dated February 11, 2002, page 7). The appellant respectfully submits, however, that additional faint bands will frequently be detected in any hybridization experiment, but the fact that a specific prominent band can be detected shows the specificity of the hybridization.

During prosecution, the Examiner cited a variety of case law for the unusual premise that the actual DNA sequence itself must be disclosed for every sequence falling within the scope of the claims, including *University of California v. Eli Lilly,* 43 USPQ2d 1398 (see appellant's previous response filed October 19, 2001 (page 8)). This is clearly in contrast to the teachings of Example 9 of the Synopsis of Application of Written Description Guidelines discussed above. Furthermore, the appellant respectfully submits that the merits of each case must be examined on a case-by-case basis, and *Lilly* does not suggest otherwise. Moreover, *Lilly* is only relevant to the particular circumstances surrounding that case, which happened to occur at a time when the art of biotechnology was much less developed than it is was at the time the present invention was made. In fact, the present application was filed after the publication of the popular Sambrook Molecular Cloning manual (2nd edition), which standardized many of the cloning procedures now used to identify and isolate genomic DNAs and cDNAs from related species. Indeed, given the existence of the Sambrook manual at the time the present invention was filed, those of skill in the art would have surely seen that the inventor was in possession of a genus of mammalian GDF-1 nucleic acids, including genomic DNAs and sequences from other species, upon reading the present disclosure.

For instance, according to the pages from Sambrook *et al.*, 1989, Molecular Cloning: A Laboratory Manual (2d edition) (Exhibit G), libraries generated from mammalian genomic DNA had been in use since the mid-1970's for cloning mammalian genes (see page 9.2). And according to the teachings on page 9.3, it was well-known at the time this Manual was published that one could use libraries of randomly cleaved DNA to "walk" along the eukaryotic

chromosome starting with a single specific probe, in order to isolate segments of DNA in and around target sequences without knowledge of the location of surrounding restriction sites.

Further, according to Sambrook *et al.* on page 8.46, nucleic acid hybridization is "the most commonly used and reliable method of screening cDNA libraries for clones of interest . . . [A]s a result of more than twenty years of work, the theoretical basis of nucleic hybridization is well-understood." In particular, partially homologous probes were commonly used at the time Sambrook *et al.* was published to detect cDNA clones that are related, but not identical, to the probe sequences. For example, if the same gene has already been cloned from another species, Sambrook *et al.* suggests performing a series of Southern hybridizations at different stringencies to determine the conditions that will allow the previously cloned gene to be used as a probe for isolating the corresponding cDNA from another species (see page 8.47). The corresponding cDNA is then isolated from a cDNA library and validated using any of the methods described on page 8.51 of Sambrook *et al.* In particular, the open reading frame of the cDNA may be sequenced to determine the corresponding amino acid sequence.

Thus, it was common practice at the time the present application was filed to isolate the genomic DNA following hybridization of a cDNA probe to a genomic library. Further, it was common practice at the time the present application was filed to isolate related cDNAs from other species using hybridization of a nucleic acid probe to a cDNA library. The Written Description Guidelines (FR, Vol. 66, No. 4, page 1099, January 5, 2001) (Exhibit H) specify that such common techniques need not be described, because one of skill in the art would be familiar with such techniques and would incorporate such knowledge into his understanding as to what the inventor possessed at the time of filing.

To illustrate, in the Federal Register publication of the Written Description Guidelines for Examination, the Office answered one comment by stating that "[a]ctual reduction to practice may be crucial in the relatively rare instances where the level of knowledge and level of skill are such that those of skill in the art cannot describe a composition structurally, or specify a process of making a composition by naming components and combining steps" (with emphasis, see page 1101). In fact, the Guidelines state at page 1106 that:

> An applicant may show that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics which provide evidence that applicant was in possession of the claimed invention, i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics. What is conventional or well known to one of ordinary skill in the art need not be disclosed in detail. If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met. (With emphasis.)

Footnote 42 of the Guidelines further defines some identifying characteristics for biomolecules to include sequence, structure, binding affinity, binding specificity, molecular weight, length, unique cleavage by particular enzymes, detailed restriction maps, a comparison of enzymatic activities, or antibody cross-reactivity (see page 1110 of the FR Notice). If binding specificity is one acceptable characteristic to be combined with sequence data and restriction maps for satisfaction of the written description requirement, then hybridization experiments showing specific hybridization of an isolated and disclosed nucleic acid sequence with a specific genomic restriction fragment should also be sufficient.

The Examiner responded to appellant's arguments by noting that appellant is arguing enablement rather than written description (Office Action, February 11, 2002, page 8). Appellant respectfully notes, however, that appellant's arguments are based on the written description guidelines promulgated by the Office, and are therefore pertinent to written description of the invention. Thus, one of skill in the art would immediately see upon reading the present disclosure that the disclosed GDF-1 coding sequences may be used as a probe to specifically identify genomic sequences and other cDNA sequences by hybridization. Further, having full knowledge of molecular biology techniques that were well-known in the art at the time the invention was made, the skilled artisan would immediately see upon viewing the

disclosed hybridization experiments that the present inventor was in possession of the genus of nucleic acids that hybridize to the disclosed GDF-1 sequences.

In view of the remarks above, the appellant submits that the specification provides defining structural features of a GDF-1 gene so as to provide support for the genus recited in claims 31-34 and 39-42, and furthermore, that the skilled artisan would immediately recognize that the appellant was in possession of the claimed genus at the time the application was filed. Thus, appellant respectfully submits that no matter what the Board decides with regard to written description of claims 22, 3 and 11-15, 22, claims 31-34 and 39-42 satisfy the requirements of the written description §112, first paragraph.

Claims 24-30 and 35-38

The Examiner has also finally rejected claims 24-30 and 35-38 under 35 U.S.C. §112, first paragraph, for allegedly containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the invention. It is pertinent to note that claims 24 and 35, and the claims dependent thereon, are directed to isolated DNAs comprising the specific open reading frame for GDF-1 that is disclosed in the specification. Thus, these claims do not read on genomic DNA sequences, given the presence of intronic sequences in the GDF-1 genomic locus as shown in Figure 1h of Ramkin et al. (Exhibit D).

According to the Office Action dated February 11, 2002 (page 6), claims 24 and 35 include sequences outside the open reading frame which have not been disclosed and are therefore not described. The appellant strongly disagrees with this assertion as the specification describes several isolated cDNA molecules comprising the open reading from for GDF-1 in addition to an upstream open reading frame as well as sequences that intervene these two open reading frames.

For instance, as described on page 28 of the specification, a region spanning 2.7 kb was cloned and sequenced from a cDNA library (sequence provided in Fig. 11a). This 2.7 kb fragment comprises not only the 1.4 kb GDF-1 open reading frame, but also a 1310 base region upstream of the GDF-1 coding region. Within this upstream region, the inventor unexpectedly

identified a second open reading frame beginning at nucleotide 74, extending for 350 codons and terminating 404 nucleotides upstream of the initiating GDF-1 ATG codon (see page 29, last paragraph). Similarly, a 2510 bp human cDNA comprising both the GDF-1 open reading frame in addition to the upstream open reading frame is also disclosed (see page 30, sequence provided in Fig. 11b). Thus, the inventor isolated cDNA nucleic acid segments that comprise the GDF-1 coding sequence in addition to upstream sequences, and this disclosure is sufficient to warrant the scope of claims 24 and 35.

In any case, notwithstanding the isolated cDNA clones specifically described the specification, claims 24, 25 and 35 are directed to isolated DNA segments comprising the specific GDF-1 nucleic acid sequences that are explicitly disclosed in the specification. Claims 26-30 and 36-38 are directed to vectors and host cells comprising such sequences, and to methods of making a recombinant GDF-1 protein using the host cells, respectively. The appellant respectfully submits that is it perfectly acceptable according to PTO policy to claim larger segments of DNA encompassing a novel DNA sequence, because a necessary common attribute of such larger sequences is the newly disclosed DNA sequence.

For instance, Example 8 of the Written Description Training Examples in the PTO's Synopsis of Application of Written Description Guidelines (p. 33, Exhibit F), describes a similar scenario where the specification discloses a novel sequence (SEQ ID NO: 2) having high homology to known DNA ligases. Claim 1 is directed to: "An isolated and purified nucleic acid comprising SEQ ID NO: 2." According to the PTO's analysis (see p. 34), it is acknowledged that the claim is drawn to any nucleic acid that minimally contains SEQ ID NO: 2, and that the claim reads on the claimed ORF in any construct or with additional nucleic acid residues placed at either end of the ORF. However, one of skill in the art "can readily envisage nucleic acid sequences which include SEQ ID NO: 2 because *e.g.* SEQ ID NO: 2 can readily be embedded in known vectors. Although there may be substantial variability among the species of DNAs encompassed within the scope of the claim because SEQ ID NO: 2 may be combined with sequences known in the art, *e.g.*, expression vectors, the necessary common attribute is the ORF" (SEQ ID NO: 2) (see sentence bridging pp. 34-35).

Thus, claims 24-30 and 35-38 are directed to nucleic acids comprising the specific GDF-1 nucleic acid sequence disclosed in the specification and methods of using the same to produce recombinant GDF-1 protein. If the Examiner's standard was to prevail, every inventor discovering a novel DNA sequence would be limited to only that novel gene sequence, and would not be able to protect the use of that sequence once it was cloned into any vector or other larger piece of DNA. The Examiner has offered no case law in support of this unusually strict position, and has not responded to appellant's request for reconsideration. Further, the Examiner's position is contradictory to Example 8 of the PTO's Written Description Training Examples discussed above. Thus, appellant respectfully submits that no matter what the Board decides with regard to written description of claims 3, 11-15, 22, 31-34 and 39-42, claims 24 and 26-30 and 35-38 satisfy the requirements of the written description §112, first paragraph.

(3) New Matter

The Examiner indicated in the Office Action dated October 21, 2002, that the rejection under 35 U.S.C. §112, first paragraph, is both a written description rejection and a new matter rejection (see page 3), presumably because appellant has allegedly failed to point to a page and line number for support for claims 39-42, as originally requested in the Office Action dated February 11, 2002 (bottom of page 7).

In maintaining this aspect of the written description rejection, the Examiner appears to have ignored the appellant's remarks on page 11 of the Reply filed July 12, 2002, providing the page and line number in support of claims 39-42. Appellant again notes that the requisite support may be found on page 10, lines 8-9, where it is disclosed that 20X SSC is defined as 3M sodium chloride/0.3M sodium citrate. So, by extension, 2X SSC would be defined as stated in claim 39. Page 9, lines 11-12 give support for washing at 68°C in 2X SSC, and page 17, lines 9-13 provide support for hybridization at 65°C.

Claims 40-42 are dependent claims directed to vectors and host cells comprising the claimed nucleic acid, and to a method of producing a recombinant protein using the claimed host cell, respectively. Support for these claims may be found in original claims 11, 12 and 15.

In view of the above remarks, the appellant respectfully submits that claims 39-42 contain no new matter, and furthermore that the claims fully satisfy the requirements of §112, first paragraph.

Summary

In summary, the appellant submits that his invention is supported by a specific, substantial and credible utility such that the skilled artisan would know how to use the claimed invention. The appellant further submits that the specification provides defining structural features of a GDF-1 gene so as to provide support for the claimed genus, and furthermore, that the skilled artisan would immediately recognize that the appellant was in possession of the claimed genus at the time the application was filed. In any case, no matter what the Board decides with regard to claims 3, 11-15, 22, 31-34 and 39-42, claims 24-30 and 35-38, which are directed to isolated sequences comprising the disclosed GDF-1 sequences, are fully supported by the specification as filed.

Accordingly, it is submitted that the Examiner's §§101 and 112 rejections of the claims are in error and should be reversed.

Respectfully submitted,

MORGAN LEWIS & BOCKIUS LLP

By _____
Bonnie Weiss McLeod
Reg. No. 43,255

1111 Pennsylvania Avenue, N.W.
Washington, D.C. 20004
Phone: (202) 739-3000
Facsimile: (202) 739-3001
Direct: (202) 739-6150

EXHIBIT A
CLAIMS ON APPEAL

22. An isolated DNA segment encoding mammalian GDF-1 protein having the amino acid sequence defined in an open reading frame of Figure 2 or Figure 11A or Figure 11B.

3. The DNA segment according to claim 22 wherein said mammal is a mouse, or human.

11. A recombinant DNA molecule comprising:

i) said DNA segment according to claim 22, operably linked to

ii) a vector.

12. A host cell stably transformed with said recombinant DNA molecule according to claim 11.

13. The host cell according to claim 12 wherein said cell is a procaryotic cell.

14. The host cell according to claim 12 wherein said cell is a eucaryotic cell.

15. A method of producing a recombinant GDF-1 protein comprising culturing the host cell of claim 12 under conditions such that said GDF-1 protein is produced, and isolating said GDF-1 protein.

24. An isolated DNA segment encoding mammalian GDF-1 protein comprising a nucleotide sequence as defined in an open reading frame of Figure 2 or Figure 11A or Figure 11B.

25. The isolated DNA segment according to claim 24 further comprising a nucleotide sequence outside the open reading frame as defined in Figure 2 or Figure 11A or Figure 11B.

26. A recombinant DNA molecule comprising the isolated DNA segment according to claim 25 operably linked to a vector.

27. A host cell stably transformed with the recombinant DNA molecule according to claim 26.

28. The host cell according to claim 27 wherein said cell is a prokaryotic cell.

29. The host cell according to claim 27 wherein said cell is a eukaryotic cell.

30. A method of producing a recombinant GDF-1 protein comprising culturing the host cell according to claim 27 under conditions such that the GDF-1 protein is produced, and isolating the GDF-1 protein.

31. An isolated DNA segment encoding mammalian GDF-1 protein which hybridizes to the nucleotide sequence defined in Figure 2 under conditions of 68°C and 1M sodium chloride and which remains bound when subjected to washing at 68°C with 15 mM sodium chloride/1.5 mM sodium citrate.

32. A recombinant DNA molecule comprising the isolated DNA segment according to claim 31 operably linked to a vector.

33. A host cell stably transformed with the recombinant DNA molecule according to claim 32.

34. A method of producing a recombinant GDF-1 protein comprising culturing the host cell according to claim 33 under conditions such that the GDF-1 protein is produced, and isolating the GDF-1 protein.

35. An isolated DNA segment encoding a mammalian GDF-1 protein which comprises the open reading frame for GDF-1 as shown in Figure 2 or Figure 11A or Figure 11B.

36. A recombinant DNA molecule comprising the isolated DNA segment according to claim 35 operably linked to a vector.

37. A host cell stably transformed with the recombinant DNA molecule according to claim 36.

38. A method of producing a recombinant GDF-1 protein comprising culturing the host cell according to claim 37 under conditions such that the GDF-1 protein is produced, and isolating the GDF-1 protein.

39. An isolated DNA segment encoding a mammalian GDF-1 protein, wherein said DNA hybridizes under conditions of 65°C and 1M sodium chloride to DNA having the nucleotide sequence as defined in Figure 2 or Figure 11A or 11B and remains bound when subjected to washing at 68°C and 0.3 M sodium chloride/ 30 mM sodium citrate (2X SSC).

40. A recombinant DNA molecule comprising the isolated DNA segment according to claim 39 operably linked to a vector.

41. A host cell stably transformed with the recombinant DNA molecule according to claim 40.

42. A method of producing a recombinant GDF-1 protein comprising culturing the host cell according to claim 41 under conditions such that the GDF-1 protein is produced, and isolating the GDF-1 protein.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#66
1063

In re Application of

LEE

Serial No.: 08/966,233

Group Art Unit: 1631

Filed: November 7, 1997

Examiner: M.P. Allen

Title: GDF-1 GENE

DATE: May 14, 2003

APPELLANT'S APPEAL BRIEF

EXHIBITS

056100-5027-US

EXHIBIT A
CLAIMS ON APPEAL

22. An isolated DNA segment encoding mammalian GDF-1 protein having the amino acid sequence defined in an open reading frame of Figure 2 or Figure 11A or Figure 11B.

3. The DNA segment according to claim 22 wherein said mammal is a mouse, or human.

11. A recombinant DNA molecule comprising:

i) said DNA segment according to claim 22, operably linked to

ii) a vector.

12. A host cell stably transformed with said recombinant DNA molecule according to claim 11.

13. The host cell according to claim 12 wherein said cell is a procaryotic cell.

14. The host cell according to claim 12 wherein said cell is a eucaryotic cell.

15. A method of producing a recombinant GDF-1 protein comprising culturing the host cell of claim 12 under conditions such that said GDF-1 protein is produced, and isolating said GDF-1 protein.

24. An isolated DNA segment encoding mammalian GDF-1 protein comprising a nucleotide sequence as defined in an open reading frame of Figure 2 or Figure 11A or Figure 11B.

25. The isolated DNA segment according to claim 24 further comprising a nucleotide sequence outside the open reading frame as defined in Figure 2 or Figure 11A or Figure 11B.

26. A recombinant DNA molecule comprising the isolated DNA segment according to claim 25 operably linked to a vector.

27. A host cell stably transformed with the recombinant DNA molecule according to claim 26.

28. The host cell according to claim 27 wherein said cell is a prokaryotic cell.

29. The host cell according to claim 27 wherein said cell is a eukaryotic cell.

30. A method of producing a recombinant GDF-1 protein comprising culturing the host cell according to claim 27 under conditions such that the GDF-1 protein is produced, and isolating the GDF-1 protein.

31. An isolated DNA segment encoding mammalian GDF-1 protein which hybridizes to the nucleotide sequence defined in Figure 2 under conditions of 68°C and 1M sodium chloride and which remains bound when subjected to washing at 68°C with 15 mM sodium chloride/1.5 mM sodium citrate.

32. A recombinant DNA molecule comprising the isolated DNA segment according to claim 31 operably linked to a vector.

33. A host cell stably transformed with the recombinant DNA molecule according to claim 32.

34. A method of producing a recombinant GDF-1 protein comprising culturing the host cell according to claim 33 under conditions such that the GDF-1 protein is produced, and isolating the GDF-1 protein.

35. An isolated DNA segment encoding a mammalian GDF-1 protein which comprises the open reading frame for GDF-1 as shown in Figure 2 or Figure 11A or Figure 11B.

36. A recombinant DNA molecule comprising the isolated DNA segment according to claim 35 operably linked to a vector.

37. A host cell stably transformed with the recombinant DNA molecule according to claim 36.

38. A method of producing a recombinant GDF-1 protein comprising culturing the host cell according to claim 37 under conditions such that the GDF-1 protein is produced, and isolating the GDF-1 protein.

39. An isolated DNA segment encoding a mammalian GDF-1 protein, wherein said DNA hybridizes under conditions of 65°C and 1M sodium chloride to DNA having the nucleotide sequence as defined in Figure 2 or Figure 11A or 11B and remains bound when subjected to washing at 68°C and 0.3 M sodium chloride/ 30 mM sodium citrate (2X SSC).

40. A recombinant DNA molecule comprising the isolated DNA segment according to claim 39 operably linked to a vector.

41. A host cell stably transformed with the recombinant DNA molecule according to claim 40.

42. A method of producing a recombinant GDF-1 protein comprising culturing the host cell according to claim 41 under conditions such that the GDF-1 protein is produced, and isolating the GDF-1 protein.

Prog Growth Factor Res 1990;2(3):153-68

B

Transforming growth factor betas in mammalian embryogenesis.

Akhurst RJ, FitzPatrick DR, Gatherer D, Lehnert SA, Millan FA.

Duncan Guthrie Institute of Medical Genetics, University of Glasgow, U.K.

Type beta transforming growth factors (TGF beta s) are members of a large superfamily of related proteins, each of which plays a pivotal role in embryonic processes. The TGF beta s per se are at least five in number, though only three isoforms have been identified in mammals. Here we will review the evidence, taken from in vitro studies on bioactivity and histochemical localization of RNAs and encoded proteins in vivo, that TGF beta 1, beta 2 and beta 3 are involved in several mammalian developmental processes, including control of growth, differentiation, tissue inductions and morphogenesis.

Publication Types:

- Review
- Review, tutorial

PMID: 2132953 [PubMed - indexed for MEDLINE]

DEPARTMENT OF COMMERCE

National Oceanic and Atmospheric Administration

Fair Market Value Analysis for a Fiber Optic Cable Permit in National Marine Sanctuaries

AGENCY: Office of National Marine Sanctuaries (ONMS), National Ocean Service (NOS), National Oceanic and Atmospheric Administration (NOAA), Department of Commerce (DOC).

ACTION: Notice of availability.

SUMMARY: Notice is hereby given that NOAA is requesting comments on the report "Fair Market Value Analysis for a Fiber Optic Cable Permit in National Marine Sanctuaries" and two peer reviews of this report. The report and peer reviews are available for download at http://www.sanctuaries.nos.noaa.gov/news/newsbboard/newsbboard.html or by requesting an electronic or hard copy. Requests can be made by sending an email to submarine.cables@noaa.gov (subject line "Request for Fair Market Value Analysis") or by calling Matt Brookhart at (301) 713–3125 x140.

DATES: Comments on this notice must be received by January 18, 2001.

ADDRESSES: Address all comments regarding this notice to Matt Brookhart, Conservation Policy and Planning Branch, Office of National Marine Sanctuaries, 1305 East-West Highway, 11th Floor, Silver Spring, MD 20910, Attention: Fair Market Value Analysis. Comments may also be submitted by email to: *submarine.cables@noaa.gov,* subject line "Fair Market Value Analysis."

FOR FURTHER INFORMATION CONTACT: Helen Golde, (301) 713–3125 x152.

SUPPLEMENTARY INFORMATION: The Office of National Marine Sanctuaries has issued several special-use permits to companies seeking to install fiber optic cables in National Marine Sanctuaries. The Sanctuary statute allows ONMS to permit the presence of cables on the sanctuaries' seafloor should it decide to do so. If an application is approved, ONMS may collect certain administrative and monitoring fees. In addition, ONMS is entitled to receive fair market value for the permitted use of sanctuary resources.

The report "Fair Market Value Analysis for a Fiber Optic Cable Permit in National Marine Sanctuaries" presents an assessment of fair market value for the use of National Marine Sanctuary resources for a fiber optic cable. Proper stewardship of sanctuary resources and open and equitable relations with telecommunication industry interests require a clear and consistent policy in this matter. The content of this report is based on dozens of industry and government sources and draws on the collaboration and review of numerous experts in the business, legal and technical arenas.

Once finalized, the fee structure proposed in this report will be used to assess fees (as stated in their respective special use permits) for cables already installed in the Olympic Coast and Stellwagen Bank National Marine Sanctuaries. In addition, this structure will provide the basis for future fair market value assessment of submarine cable permit applications in National Marine Sanctuaries. Comments on the report and peer reviews should focus on the methodology employed and the conclusions that it reached.

Dated: December 29, 2000.

John Oliver,

Chief Financial Officer, National Ocean Service.

[FR Doc. 01–387 Filed 1–4–01; 8:45 am]

BILLING CODE 3510–08–P

DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

[Docket No. 991027289–0263–02]

RIN 0651–AB09

Utility Examination Guidelines

AGENCY: United States Patent and Trademark Office, Commerce.

ACTION: Notice.

SUMMARY: The United States Patent and Trademark Office (USPTO) is publishing a revised version of guidelines to be used by Office personnel in their review of patent applications for compliance with the "utility" requirement of 35 U.S.C. 101. This revision supersedes the Revised Interim Utility Examination Guidelines that were published at 64 FR 71440, Dec. 21, 1999; 1231 O.G. 136 (2000); and correction at 65 FR 3425, Jan. 21, 2000; 1231 O.G. 67 (2000).

DATES: The Guidelines are effective as of January 5, 2001.

FOR FURTHER INFORMATION CONTACT: Mark Nagumo by telephone at (703) 305–8666, by facsimile at (703) 305–9373, by electronic mail at "mark.nagumo@uspto.gov," or by mail marked to his attention addressed to the Office of the Solicitor, Box 8, Washington, DC 20231; or Linda Therkorn by telephone at (703) 305–9323, by facsimile at (703) 305–8825, by electronic mail at "linda.therkorn@uspto.gov," or by mail marked to her attention addressed to Box Comments, Commissioner for Patents, Washington, DC 20231.

SUPPLEMENTARY INFORMATION: As of the publication date of this notice, these Guidelines will be used by USPTO personnel in their review of patent applications for compliance with the "utility" requirement of 35 U.S.C. 101. Because these Guidelines only govern internal practices, they are exempt from notice and comment rulemaking under 5 U.S.C. 553(b)(A).

I. Discussion of Public Comments

The Revised Interim Utility Examination Guidelines published at 64 FR 71440, Dec. 21, 1999; 1231 O.G. 136, Feb. 29, 2000, with a correction at 65 FR 3425, Jan. 21, 2000; 1231 O.G. 67, Feb. 15, 2000, requested comments from the public. Comments were received from 35 individuals and 17 organizations. The written comments have been carefully considered.

Overview of Comments

The majority of comments generally approved of the guidelines and several expressly stated support for the three utility criteria (specific, substantial, and credible) set forth in the Guidelines. A few comments addressed particular concerns with respect to the coordinate examiner training materials that are available for public inspection at the USPTO website, *www.uspto.gov.* The comments on the training materials will be taken under advisement in the revision of the training materials. Consequently, those comments are not specifically addressed below because they do not impact the content of the Guidelines. Comments received in response to the request for comments on the "Revised Interim Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶1 'Written Description' Requirement," 64 FR 71427, Dec. 21, 1999; 1231 O.G. 123, Feb. 29, 2000, which raised issues pertinent to the utility requirement are also addressed below.

Responses to Specific Comments

(1) *Comment:* Several comments state that while inventions are patentable, discoveries are not patentable. According to the comments, genes are discoveries rather than inventions. These comments urge the USPTO not to issue patents for genes on the ground that genes are not inventions. *Response:* The suggestion is not adopted. An inventor can patent a discovery when the patent application satisfies the statutory requirements. The U.S.

Constitution uses the word "discoveries" where it authorizes Congress to promote progress made by inventors. The pertinent part of the Constitution is Article 1, section 8, clause 8, which reads: "The Congress shall have power * * * To promote the Progress of Science and useful Arts, by securing for limited Times to Authors and Inventors the exclusive Right to their respective Writings and Discoveries."

When Congress enacted the patent statutes, it specifically authorized issuing a patent to a person who "invents or discovers" a new and useful composition of matter, among other things. The pertinent statute is 35 U.S.C. 101, which reads: "Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title." Thus, an inventor's discovery of a gene can be the basis for a patent on the genetic composition isolated from its natural state and processed through purifying steps that separate the gene from other molecules naturally associated with it.

If a patent application discloses only nucleic acid molecular structure for a newly discovered gene, and no utility for the claimed isolated gene, the claimed invention is not patentable. But when the inventor also discloses how to use the purified gene isolated from its natural state, the application satisfies the "utility" requirement. That is, where the application discloses a specific, substantial, and credible utility for the claimed isolated and purified gene, the isolated and purified gene composition may be patentable.

(2) *Comment:* Several comments state that a gene is not a new composition of matter because it exists in nature, and/or that an inventor who isolates a gene does not actually invent or discover a patentable composition because the gene exists in nature. These comments urge the USPTO not to issue patents for genes on the ground that genes are products of nature. Others state that naturally occurring DNAs are part of our heritage and are not inventions. Another comment expressed concern that a person whose body includes a patented gene could be guilty of patent infringement. *Response:* The comments are not adopted. A patent claim directed to an isolated and purified DNA molecule could cover, *e.g.*, a gene excised from a natural chromosome or a synthesized DNA molecule. An isolated and purified DNA molecule that has the same sequence as a naturally occurring gene is eligible for a patent because (1) an excised gene is eligible for a patent as a composition of matter or as an article of manufacture because that DNA molecule does not occur in that isolated form in nature, or (2) synthetic DNA preparations are eligible for patents because their purified state is different from the naturally occurring compound.

Patenting compositions or compounds isolated from nature follows well-established principles, and is not a new practice. For example, Louis Pasteur received U.S. Patent 141,072 in 1873, claiming "[y]least, free from organic germs of disease, as an article of manufacture." Another example is an early patent for adrenaline. In a decision finding the patent valid, the court explained that compounds isolated from nature are patentable: "even if it were merely an extracted product without change, there is no rule that such products are not patentable. Takamine was the first to make it [adrenaline] available for any use by removing it from the other gland-tissue in which it was found, and, while it is of course possible logically to call this a purification of the principle, it became for every practical purpose a new thing commercially and therapeutically. That was a good ground for a patent." *Parke-Davis & Co.* v. *H. K. Mulford Co.*, 189 F. 95, 103 (S.D.N.Y. 1911) (J. Learned Hand).

In a more recent case dealing with the prostaglandins PGE_2 and PGE_3, extracted from human or animal prostate glands, a patent examiner had rejected the claims, reasoning that "inasmuch as the 'claimed compounds are naturally occurring' * * * they therefore 'are not 'new' within the connotation of the patent statute.'" *In re Bergstrom*, 427 F.2d 1394, 1397, 166 USPQ 256, 259 (CCPA 1970). The Court reversed the Patent Office and explained the error: "what appellants claim—pure PGE_2 and PGE_3—is not 'naturally occurring.' Those compounds, as far as the record establishes, do not exist in nature in pure form, and appellants have neither merely discovered, nor claimed sufficiently broadly to encompass, what has previously existed in fact in nature's storehouse, albeit unknown, or what has previously been known to exist." *Id.* at 1401, 166 USPQ at 261–62. Like other chemical compounds, DNA molecules are eligible for patents when isolated from their natural state and purified or when synthesized in a laboratory from chemical starting materials.

A patent on a gene covers the isolated and purified gene but does not cover the gene as it occurs in nature. Thus, the concern that a person whose body "includes" a patented gene could infringe the patent is misfounded. The body does not contain the patented, isolated and purified gene because genes in the body are not in the patented, isolated and purified form. When the patent issued for purified adrenaline about one hundred years ago, people did not infringe the patent merely because their bodies naturally included unpurified adrenaline.

(3) *Comment:* Several comments suggested that the USPTO should seek guidance from Congress as to whether naturally occurring genetic sequences are patentable subject matter. *Response:* The suggestion is not adopted. Congress adopted the current statute defining patentable subject matter (35 U.S.C. 101) in 1952. The legislative history indicates that Congress intended "anything under the sun that is made by man" to be eligible for patenting. S. Rep. No. 1979, 82d Cong., 2d Sess., 5 (1952); H.R. Rep. No. 1923, 82d Cong., 2d Sess., 6 (1952). The Supreme Court interprets the statute to cover a "nonnaturally occurring manufacture or composition of matter—a product of human ingenuity." *Diamond* v. *Chakrabarty*, 447 U.S. 303, 309, 206 USPQ 193, 197 (1980). Thus, the intent of Congress with regard to patent eligibility for chemical compounds has already been determined: DNA compounds having naturally occurring sequences are eligible for patenting when isolated from their natural state and purified, and when the application meets the statutory criteria for patentability. The genetic sequence data represented by strings of the letters A, T, C and G alone is raw, fundamental sequence data, i.e., nonfunctional descriptive information. While descriptive sequence information alone is not patentable subject matter, a new and useful purified and isolated DNA compound described by the sequence is eligible for patenting, subject to satisfying the other criteria for patentability.

(4) *Comment:* Several comments state that patents should not issue for genes because the sequence of the human genome is at the core of what it means to be human and no person should be able to own/control something so basic. Other comments stated that patents should be for marketable inventions and not for discoveries in nature. *Response:* The comments are not adopted. Patents do not confer ownership of genes, genetic information, or sequences. The patent system promotes progress by securing a complete disclosure of an invention to the public, in exchange for the inventor's legal right to exclude other people from making, using, offering for sale, selling, or importing

the composition for a limited time. That is, a patent owner can stop infringing activity by others for a limited time.

Discoveries from nature have led to marketable inventions in the past, but assessing the marketability of an invention is not pertinent to determining if an invention has a specific, substantial, and credible use. "[D]evelopment of a product to the extent that it is presently commercially salable in the marketplace is not required to establish 'usefulness' within the meaning of § 101." *In re Langer,* 503 F.2d 1380, 1393, 183 USPQ 288, 298 (CCPA 1974). Inventors are entitled to patents when they have met the statutory requirements for novelty, nonobviousness and usefulness, and their patent disclosure adequately describes the invention and clearly teaches others how to make and use the invention. The utility requirement, as explained by the courts, only requires that the inventor disclose a practical or real world benefit available from the invention, i.e., a specific, substantial and credible utility. As noted in a response to other comments, it is a long tradition in the United States that discoveries from nature which are transformed into new and useful products are eligible for patents.

(5) *Comment:* Several comments state that the Guidelines mean that anyone who discovers a gene will be allowed a broad patent covering any number of possible applications even though those uses may be unattainable and unproven. Therefore, according to these comments, gene patents should not be issued. *Response:* The comment is not adopted. When a patent claiming a new chemical compound issues, the patentee has the right to exclude others from making, using, offering for sale, selling, or importing the compound for a limited time. The patentee is required to disclose only one utility, that is, teach others how to use the invention in at least one way. The patentee is not required to disclose all possible uses, but promoting the subsequent discovery of other uses is one of the benefits of the patent system. When patents for genes are treated the same as for other chemicals, progress is promoted because the original inventor has the possibility to recoup research costs, because others are motivated to invent around the original patent, and because a new chemical is made available as a basis for future research. Other inventors who develop new and nonobvious methods of using the patented compound have the opportunity to patent those methods.

(6) *Comment:* One comment suggests that the USPTO should not allow the patenting of ESTs because it is contrary to indigenous law, because the Supreme Court's *Diamond* v. *Chakrabarty* decision was a bare 5-to-4 decision, because it would violate the Thirteenth Amendment of the U.S. Constitution, because it violates the novelty requirement of the patent laws, because it will exacerbate tensions between indigenous peoples and western academic/research communities and because it will undermine indigenous peoples' own research and academic institutions. The comment urges the USPTO to institute a moratorium on patenting of life forms and natural processes. *Response:* The comments are not adopted. Patents on chemical compounds such as ESTs do not implicate the Thirteenth Amendment. The USPTO must administer the patent statutes as the Supreme Court interprets them. When Congress enacted § 101, it indicated that "anything under the sun that is made by man" is subject matter for a patent. S. Rep. No. 1979, 82d Cong., 2d Sess., 5 (1952); H.R. Rep. No. 1923, 82d Cong., 2d Sess., 6 (1952). The Supreme Court has interpreted § 101 many times without overturning it. *See, e.g., Diamond* v. *Diehr,* 450 U.S. 175, 209 USPQ 1 (1981) (discussing cases construing section 101). Under United States law, a patent applicant is entitled to a patent when an invention meets the patentability criteria of title 35. Thus, ESTs which meet the criteria for utility, novelty, and nonobviousness are eligible for patenting when the application teaches those of skill in the art how to make and use the invention.

(7) *Comment:* Several comments state that patents should not issue for genes because patents on genes are delaying medical research and thus there is no societal benefit associated with gene patents. Others state that granting patents on genes at any stage of research deprives others of incentives and the ability to continue exploratory research and development. Some comment that patentees will deny access to genes and our property (our genes) will be owned by others. *Response:* The comments are not adopted. The incentive to make discoveries and inventions is generally spurred, not inhibited, by patents. The disclosure of genetic inventions provides new opportunities for further development. The patent statutes provide that a patent must be granted when at least one specific, substantial and credible utility has been disclosed, and the application satisfies the other statutory requirements. As long as one specific, substantial and credible use is disclosed and the statutory requirements are met, the USPTO is not authorized to withhold the patent until another, or better, use is discovered. Other researchers may discover higher, better or more practical uses, but they are advantaged by the starting point that the original disclosure provides. A patent grants exclusionary rights over a patented composition but does not grant ownership of the composition. Patents are not issued on compositions in the natural environment but rather on isolated and purified compositions.

(8) *Comment:* Several comments stated that DNA should be considered unpatentable because a DNA sequence by itself has little utility. *Response:* A DNA sequence—*i.e.,* the sequence of base pairs making up a DNA molecule—is simply one of the properties of a DNA molecule. Like any descriptive property, a DNA sequence itself is not patentable. A purified DNA *molecule* isolated from its natural environment, on the other hand, is a chemical compound and is patentable if all the statutory requirements are met. An isolated and purified DNA molecule may meet the statutory utility requirement if, *e.g.,* it can be used to produce a useful protein or it hybridizes near and serves as a marker for a disease gene. Therefore, a DNA molecule is not *per se* unpatentable for lack of utility, and each application claim must be examined on its own facts.

(9) *Comment:* One comment states that the disclosure of a DNA sequence has inherent value and that possible uses for the DNA appear endless, even if no single use has been worked out. According to the comment, the "basic social contract of the patent deal" requires that such a discovery should be patentable, and that patenting should be "value-blind." *Response:* The comment is not adopted. The Supreme Court did not find a similar argument persuasive in *Brenner* v. *Manson,* 383 U.S. 519 (1966). The courts interpret the statutory term "useful" to require disclosure of at least one available practical benefit to the public. The Guidelines reflect this determination by requiring the disclosure of at least one specific, substantial, and credible utility. If no such utility is disclosed or readily apparent from an application, the Office should reject the claim. The applicant may rebut the Office position by showing that the invention does have a specific, substantial, and credible utility that would have been recognized by one of skill in the art at the time the application was filed.

(10) *Comment:* Several comments stated that the scope of patent claims directed to DNA should be limited to applications or methods of using DNA, and should not be allowed to

encompass the DNA itself. *Response:* The comment is not adopted. Patentable subject matter includes both "process[es]" and "composition[s] of matter." 35 U.S.C. 101. Patent law provides no basis for treating DNA differently from other chemical compounds that are compositions of matter. If a patent application claims a composition of matter comprising DNA, and the claims meet all the statutory requirements of patentability, there is no legal basis for rejecting the application.

(11) *Comment:* Several comments stated that DNA patent claim scope should be limited to uses that are disclosed in the patent application and that allowing patent claims that encompass DNA itself would enable the inventor to assert claims to "speculative" uses of the DNA that were not foreseen at the time the patent application was filed. *Response:* The comment is not adopted. A patent on a composition gives *exclusive* rights to the composition for a limited time, even if the inventor disclosed only a single use for the composition. Thus, a patent granted on an isolated and purified DNA composition confers the right to exclude others from *any* method of using that DNA composition, for up to 20 years from the filing date. This result flows from the language of the statute itself. When the utility requirement and other requirements are satisfied by the application, a patent granted provides a patentee with the right to exclude others from, *inter alia*, "using" the patented composition of matter. *See* 35 U.S.C. 154. Where a new use is discovered for a patented DNA composition, that new use may qualify for its own process patent, notwithstanding that the DNA composition itself is patented.

By statute, a patent is required to disclose one practical utility. If a well-established utility is readily apparent, the disclosure is deemed to be implicit. If an application fails to disclose one specific, substantial, and credible utility, and the examiner discerns no well-established utility, the examiner will reject the claim under section 101. The rejection shifts the burden to the applicant to show that the examiner erred, or that a well-established utility would have been readily apparent to one of skill in the art. The applicant cannot rebut the rejection by relying on a utility that would not have been readily apparent at the time the application was filed. *See, e.g., In re Wright*, 999 F.2d 1557, 1562–63, 27 USPQ2d 1510, 1514 (Fed. Cir. 1993) ("developments occurring after the filing date of an application are of no significance regarding what one skilled in the art believed as of the filing date").

(12) *Comment:* Several comments stated that DNA should be freely available for research. Some of these comments suggested that patents are not necessary to encourage additional discovery and sequencing of genes. Some comments suggested that patenting of DNA inhibits biomedical research by allowing a single person or company to control use of the claimed DNA. Another comment expressed concern that patenting ESTs will impede complete characterization of genes and delay or restrict exploration of genetic materials for the public good. *Response:* The scope of subject matter that is eligible for a patent, the requirements that must be met in order to be granted a patent, and the legal rights that are conveyed by an issued patent, are all controlled by statutes which the USPTO must administer. "Whoever invents or discovers any new and useful * * * composition of matter * * * may obtain a patent therefor." 35 U.S.C. 101. Congress creates the law and the Federal judiciary interprets the law. The USPTO must administer the laws as Congress has enacted them and as the Federal courts have interpreted them. Current law provides that when the statutory patentability requirements are met, there is no basis to deny patent applications claiming DNA compositions, or to limit a patent's scope in order to allow free access to the use of the invention during the patent term.

(13) *Comment:* Several comments suggested that DNA sequences should be considered unpatentable because sequencing DNA has become so routine that determining the sequence of a DNA molecule is not inventive. *Response:* The comments are not adopted. A DNA sequence is not patentable because a sequence is merely descriptive information about a molecule. An isolated and purified DNA molecule may be patentable because a molecule is a "composition of matter," one of the four classes of invention authorized by 35 U.S.C. 101. A DNA molecule must be *nonobvious* in order to be patentable. Obviousness does not depend on the amount of work required to characterize the DNA molecule. *See* 35 U.S.C. 103(a) ("Patentability shall not be negatived by the manner in which the invention was made."). As the nonobviousness requirement has been interpreted by the U.S. Court of Appeals for the Federal Circuit, whether a claimed DNA molecule would have been obvious depends on whether a molecule having the particular *structure* of the DNA would have been obvious to one of ordinary skill in the art at the time the invention was made. *See, e.g., In re Deuel*, 51 F.3d 1552, 1559, 34 USPQ2d 1210, 1215 (Fed. Cir. 1995) ("[T]he existence of a general method of isolating cDNA or DNA molecules is essentially irrelevant to the question whether the specific molecules themselves would have been obvious."); *see also, In re Bell*, 991 F.2d 781, 26 USPQ2d 1529 (Fed. Cir. 1993).

(14) *Comment:* One comment suggested that genes ought to be patentable only when the complete sequence of the gene is disclosed and a function for the gene product has been determined. *Response:* The suggestion is not adopted. To obtain a patent on a chemical compound such as DNA, a patent applicant must adequately describe the compound and must disclose how to make and use the compound. 35 U.S.C. 101, 112. "An adequate written description of a DNA * * * requires a precise definition, *such as* by structure, formula, chemical name, or physical properties." *Univ. of California* v. *Eli Lilly & Co.*, 119 F.3d 1559, 1556, 43 USPQ2d 1398, 1404 (Fed. Cir. 1997) (emphasis added, internal quote omitted). Thus, describing the complete chemical structure, *i.e.*, the DNA sequence, is one method of describing a DNA molecule but it is not the only method. In addition, the utility of a claimed DNA does not necessarily depend on the function of the encoded gene product. A claimed DNA may have a specific and substantial utility because, *e.g.*, it hybridizes near a disease-associated gene or it has a gene-regulating activity.

(15) *Comment:* One comment stated that the specification should "disclose the invention," including why the invention works and how it was developed. *Response:* The comment is not adopted. The comment is directed more to the requirements imposed by 35 U.S.C. 112 than to those of 35 U.S.C. 101. To satisfy the enablement requirement of 35 U.S.C. 112, ¶ 1, an application must disclose the claimed invention in sufficient detail to enable a person of ordinary skill in the art to make and use the claimed invention. To satisfy the written description requirement of 35 U.S.C. 112, ¶ 1, the description must show that the applicant was in possession of the claimed invention at the time of filing. If all the requirements under 35 U.S.C. 112, ¶1, are met, there is no statutory basis to require disclosure of why an invention works or how it was developed. "[I]t is not a requirement of patentability that an inventor correctly set forth, or even know, how or why the invention works." *Newman* v. *Quigg*,

877 F.2d 1575, 1581, 11 USPQ2d 1340, 1345 (Fed. Cir. 1989).

(16) *Comment:* One comment suggested that patents should "allow for others to learn from and improve the invention." The comment suggested that claims to patented plant varieties should not prohibit others from using the patented plants to develop improved varieties. The comment also stated that uses of plants in speculative manners should not be permitted. *Response:* By statute, a patent provides the patentee with the right to exclude others from, *inter alia,* making and using the claimed invention, although a limited research exemption exists. *See* 35 U.S.C. 163, 271(a), (e). These statutory provisions are not subject to revision by the USPTO and are not affected by these Guidelines. Where a plant is claimed in a utility patent application, compliance with the statutory requirements for utility under 35 U.S.C. 101 only requires that a claimed invention be supported by at least one specific, substantial and credible utility. It is somewhat rare for academic researchers to be sued by commercial patent owners for patent infringement. Most inventions are made available to academic researchers on very favorable licensing terms, which enable them to continue their research.

(17) *Comment:* Two comments suggested that although the USPTO has made a step in the right direction in raising the bar in the Utility Guidelines, there is still a need to apply stricter standards for utility. *Response:* The USPTO is bound by 35 U.S.C. 101 and the case law interpreting § 101. The Guidelines reflect the USPTO's understanding of § 101.

(18) *Comment:* Several comments addressed specific concerns about the examiner training materials. *Response:* The comments received with respect to the training materials will be taken under advisement as the Office revises the training materials. Except for comments with regard to whether sequence homology is sufficient to demonstrate a specific and substantial credible utility, specific concerns about the training materials will not be addressed herein as they will not impact the language of the guidelines.

(19) *Comment:* Several comments suggested that the use of computer-based analysis of nucleic acids to assign a function to a given nucleic acid based upon homology to prior art nucleic acids found in databases is highly unpredictable and cannot form a basis for an assignment of function to a putatively encoded protein. These comments also indicate that even in instances where a general functional assignment may be reasonable, the assignment does not provide information regarding the actual biological activity of an encoded protein and therefore patent claims drawn to such nucleic acids should be limited to method of use claims that are explicitly supported by the as-filed specification(s). These comments also state that if homology-based utilities are acceptable, then the nucleic acids, and proteins encoded thereby, should be considered as obvious over the prior art nucleic acids. On the other hand, one comment stated that homology is a standard, art-accepted basis for predicting utility, while another comment stated that any level of homology to a protein with known utility should be accepted as indicative of utility. *Response:* The suggestions to adopt a *per se* rule rejecting homology-based assertions of utility are not adopted. An applicant is entitled to a patent to the subject matter claimed unless statutory requirements are not met (35 U.S.C. 101, 102, 103, 112). When the USPTO denies a patent, the Office must set forth at least a *prima facie* case as to why an applicant has not met the statutory requirements. The inquiries involved in assessing utility are fact dependent, and the determinations must be made on the basis of scientific evidence. Reliance on the commenters' *per se* rule, rather than a fact dependent inquiry, is impermissible because the commenters provide no scientific evidence that homology-based assertions of utility are inherently unbelievable or involve implausible scientific principles. *See, e.g., In re Brana,* 51 F.3d 1560, 1566, 34 USPQ2d 1436, 1441 (Fed. Cir. 1995) (rejection of claims improper where claims did "not suggest an inherently unbelievable undertaking or involve implausible scientific principles' and where "prior art * * * discloses structurally similar compounds to those claimed by the applicants which have been proven * * * to be effective").

A patent examiner must accept a utility asserted by an applicant unless the Office has evidence or sound scientific reasoning to rebut the assertion. The examiner's decision must be supported by a preponderance of all the evidence of record. *In re Oetiker,* 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). More specifically, when a patent application claiming a nucleic acid asserts a specific, substantial, and credible utility, and bases the assertion upon homology to existing nucleic acids or proteins having an accepted utility, the asserted utility must be accepted by the examiner unless the Office has sufficient evidence or sound scientific reasoning to rebut such an assertion. "[A] 'rigorous correlation' need not be shown in order to establish practical utility; 'reasonable correlation' is sufficient." *Fujikawa* v. *Wattanasin,* 93 F.3d 1559, 1565, 39 USPQ2d 1895, 1900 (Fed. Cir. 1996). The Office will take into account both the nature and degree of the homology.

When a class of proteins is defined such that the members share a specific, substantial, and credible utility, the reasonable assignment of a new protein to the class of sufficiently conserved proteins would impute the same specific, substantial, and credible utility to the assigned protein. If the preponderance of the evidence of record, or of sound scientific reasoning, casts doubt upon such an asserted utility, the examiner should reject the claim for lack of utility under 35 U.S.C. 101. For example, where a class of proteins is defined by common structural features, but evidence shows that the members of the class do not share a specific, substantial functional attribute or utility, despite having structural features in common, membership in the class may not impute a specific, substantial, and credible utility to a new member of the class. When there is a reason to doubt the functional protein assignment, the utility examination may turn to whether or not the asserted protein encoded by a claimed nucleic acid has a well-established use. If there is a well-established utility for the protein and the claimed nucleic acid, the claim would meet the requirements for utility under 35 U.S.C. 101. If not, the burden shifts to the applicant to provide evidence supporting a well-established utility. There is no *per se* rule regarding homology, and each application must be judged on its own merits.

The comment indicating that if a homology-based utility could meet the requirements set forth under 35 U.S.C. 101, then the invention would have been obvious, is not adopted. Assessing nonobviousness under 35 U.S.C. 103 is separate from analyzing the utility requirements under 35 U.S.C. 101. When a claim to a nucleic acid supported by a homology-based utility meets the utility requirement of section 101, it does not follow that the claimed nucleic acid would have been *prima facie* obvious over the nucleic acids to which it is homologous. "[S]ection 103 requires a fact-intensive comparison of the [claim] with the prior art rather than the mechanical application of one or another *per se* rule." *In re Ochiai,* 71 F.3d 1565, 1571, 37 USPQ2d 1127, 1132 (Fed. Cir. 1995). Nonobviousness must be determined according to the analysis

in *Graham* v. *John Deere*, 383 U.S. 1, 148 USPQ 459 (1966). *See also, In re Dillon*, 919 F.2d 688, 692, 16 USPQ2d 1897, 1901 (Fed. Cir. 1990) (in banc) ("structural similarity between claimed and prior art subject matter, * * * *where the prior art gives reason or motivation to make the claimed compositions*, creates a prima facie case of obviousness") (emphasis added). Where "the prior art teaches a specific, structurally-definable compound [] the question becomes whether the prior art would have suggested making the specific molecular modifications necessary to achieve the claimed invention." *In re Deuel*, 51 F.3d 1552, 1558, 34 USPQ2d 1210, 1214 (Fed. Cir. 1995).

(20) *Comment:* Several comments indicated that in situations where a well-established utility is relied upon for compliance with 35 U.S.C. 101, the record should reflect what that utility is. One comment stated that the record should reflect whether the examiner accepted an asserted utility or relied upon a well-established utility after dismissing all asserted utilities. Another comment stated that when the examiner relies on a well-established utility not explicitly asserted by the applicant, the written record should clearly identify this utility and the rationale for considering it specific and substantial. *Response:* The comments are not adopted. Only one specific, substantial and credible utility is required to satisfy the statutory requirement. Where one or more well-established utilities would have been readily apparent to those of skill in the art at the time of the invention, an applicant may rely on any one of those utilities without prejudice. The record of any issued patent typically reflects consideration of a number of references in the prior art that the applicant or the examiner considered material to the claimed invention. These references often indicate uses for related inventions, and any patents listed typically disclose utilities for related inventions. Thus, even when the examiner does not identify a well-established utility, the record as a whole will likely disclose readily apparent utilities. Just as the examiner without comment may accept a properly asserted utility, there is no need for an examiner to comment on the existence of a well-established utility. However, the Guidelines have been revised to clarify that a well-established utility is a specific, substantial, and credible utility that must be readily apparent to one skilled in the art. Most often, the closest prior art cited and applied in the course of examining the application will demonstrate a well-established utility for the invention.

(21) *Comment:* Several comments stated that the Guidelines erroneously burden the examiner with proving that a person of skill in the art would not be aware of a well-established utility. One comment states that this requires the examiner to prove a negative. Another comment states that the Guidelines should direct examiners that if a specific utility has not been disclosed, the applicant should be required to identify a specific utility. *Response:* The comments have been adopted in part. The Guidelines have been revised to indicate that where the applicant has not asserted a specific, substantial, and credible utility, and the examiner does not perceive a well-established utility, a rejection under § 101 should be entered. That is, if a well-established utility is not readily apparent and an invention is not otherwise supported by an asserted specific, substantial, and credible utility, the burden will be shifted to applicant to show either that the specification discloses an adequate utility, or to show that a well-established utility exists for the claimed invention. Again, most often the search of the closest prior art will reveal whether there is a well-established utility for the claimed invention.

(22) *Comment:* Several comments suggested that further clarification was required with regard to the examiner's determination that there is an adequate nexus between a showing supporting a well-established utility and the application as filed. The comments indicated that the meaning of this "nexus" was unclear. *Response:* The Guidelines have been modified to reflect that evidence provided by an applicant is to be analyzed with regard to a concordance between the showing and the full scope and content of the claimed invention as disclosed in the application as filed. In situations where the showing provides adequate evidence that the claim is supported by at least one asserted specific, substantial, and credible or well-established utility, the rejections under 35 U.S.C. 101 and 112, first paragraph, will be withdrawn. However, the examiner is instructed to consider whether or not the specification, in light of applicant's showing, is enabled for the use of the full scope of the claimed invention. Many times prior patents and printed publications provided by applicant will clearly demonstrate that a well-established utility exists.

(23) *Comment:* One comment states that the Office is using an improper standard in assessing "specific" utility. According to the comment, a distinction between "specific" and "general" utilities is an overreaching interpretation of the specificity requirement in the case law because "unique" or "particular" utilities have never been required by the law. The comment states that the specificity requirement concerns sufficiency of disclosure, *i.e.*, teaching how to make and use a claimed invention, not the utility requirement. The comment states that the specificity requirement is to be distinguished from the "substantial" utility requirement, and that the *Brenner* v. *Manson* decision concerned only a "substantial" utility issue, not specificity. *Response:* The comment is not adopted. The disclosure of only a general utility rather than a particular utility is insufficient to meet statutory requirements. Although the specificity requirement is relevant to § 112, it is not severable from the utility requirement.

> [S]urely Congress intended § 112 to presuppose *full satisfaction* of the requirements of § 101. Necessarily, compliance with § 112 requires a description of how to use presently useful inventions, otherwise an applicant would anomalously be required to teach how to use a useless invention. As this court stated in *Diederich*, quoting with approval from the decision of the board:
>
> 'We do not believe that it was the intention of the statutes to require the Patent Office, the courts, or the public to play the sort of guessing game that might be involved if an applicant could satisfy the requirements of the statutes by indicating the usefulness of a claimed compound in terms of possible use so general as to be meaningless and then, after his research or that of his competitors has definitely ascertained an actual use for the compound, adducing evidence intended to show that a particular specific use would have been obvious to men skilled in the particular art to which this use relates.' As the Supreme Court said in Brenner v. Manson:
>
> '* * * a patent is not a hunting license. It is not a reward for the search, but compensation for its successful conclusion.'
>
> *In re Kirk*, 376 F.2d 936, 942, 153 USPQ 48, 53 (CCPA 1967) (affirming rejections under §§ 101 and 112) (emphasis in original).

II. Guidelines for Examination of Applications for Compliance With the Utility Requirement

A. Introduction

The following Guidelines establish the policies and procedures to be followed by Office personnel in the evaluation of any patent application for compliance with the utility requirements of 35 U.S.C. 101 and 112. These Guidelines have been promulgated to assist Office personnel in their review of applications for compliance with the utility

requirement. The Guidelines do not alter the substantive requirements of 35 U.S.C. 101 and 112, nor are they designed to obviate the examiner's review of applications for compliance with all other statutory requirements for patentability. The Guidelines do not constitute substantive rulemaking and hence do not have the force and effect of law. Rejections will be based upon the substantive law, and it is these rejections which are appealable. Consequently, any perceived failure by Office personnel to follow these Guidelines is neither appealable nor petitionable.

B. Examination Guidelines for the Utility Requirement

Office personnel are to adhere to the following procedures when reviewing patent applications for compliance with the "useful invention" ("utility") requirement of 35 U.S.C. 101 and 112, first paragraph.

1. Read the claims and the supporting written description.

(a) Determine what the applicant has claimed, noting any specific embodiments of the invention.

(b) Ensure that the claims define statutory subject matter (*i.e.*, a process, machine, manufacture, composition of matter, or improvement thereof).

(c) If at any time during the examination, it becomes readily apparent that the claimed invention has a well-established utility, do not impose a rejection based on lack of utility. An invention has a well-established utility (1) if a person of ordinary skill in the art would immediately appreciate why the invention is useful based on the characteristics of the invention (*e.g.*, properties or applications of a product or process), and (2) the utility is specific, substantial, and credible.

2. Review the claims and the supporting written description to determine if the applicant has asserted for the claimed invention any specific and substantial utility that is credible:

(a) If the applicant has asserted that the claimed invention is useful for any particular practical purpose (*i.e.*, it has a "specific and substantial utility") and the assertion would be considered credible by a person of ordinary skill in the art, do not impose a rejection based on lack of utility.

(1) A claimed invention must have a specific and substantial utility. This requirement excludes "throw-away," "insubstantial," or "nonspecific" utilities, such as the use of a complex invention as landfill, as a way of satisfying the utility requirement of 35 U.S.C. 101.

(2) Credibility is assessed from the perspective of one of ordinary skill in the art in view of the disclosure and any other evidence of record (*e.g.*, test data, affidavits or declarations from experts in the art, patents or printed publications) that is probative of the applicant's assertions. An applicant need only provide one credible assertion of specific and substantial utility for each claimed invention to satisfy the utility requirement.

(b) If no assertion of specific and substantial utility for the claimed invention made by the applicant is credible, and the claimed invention does not have a readily apparent well-established utility, reject the claim(s) under § 101 on the grounds that the invention as claimed lacks utility. Also reject the claims under § 112, first paragraph, on the basis that the disclosure fails to teach how to use the invention as claimed. The § 112, first paragraph, rejection imposed in conjunction with a § 101 rejection should incorporate by reference the grounds of the corresponding § 101 rejection.

(c) If the applicant has not asserted any specific and substantial utility for the claimed invention and it does not have a readily apparent well-established utility, impose a rejection under § 101, emphasizing that the applicant has not disclosed a specific and substantial utility for the invention. Also impose a separate rejection under § 112, first paragraph, on the basis that the applicant has not disclosed how to use the invention due to the lack of a specific and substantial utility. The §§ 101 and 112 rejections shift the burden of coming forward with evidence to the applicant to:

(1) Explicitly identify a specific and substantial utility for the claimed invention; and

(2) Provide evidence that one of ordinary skill in the art would have recognized that the identified specific and substantial utility was well established at the time of filing. The examiner should review any subsequently submitted evidence of utility using the criteria outlined above. The examiner should also ensure that there is an adequate nexus between the evidence and the properties of the now claimed subject matter as disclosed in the application as filed. That is, the applicant has the burden to establish a probative relation between the submitted evidence and the originally disclosed properties of the claimed invention.

3. Any rejection based on lack of utility should include a detailed explanation why the claimed invention has no specific and substantial credible utility. Whenever possible, the examiner should provide documentary evidence regardless of publication date (*e.g.*, scientific or technical journals, excerpts from treatises or books, or U.S. or foreign patents) to support the factual basis for the *prima facie* showing of no specific and substantial credible utility. If documentary evidence is not available, the examiner should specifically explain the scientific basis for his or her factual conclusions.

(a) Where the asserted utility is not specific or substantial, a *prima facie* showing must establish that it is more likely than not that a person of ordinary skill in the art would not consider that any utility asserted by the applicant would be specific and substantial. The *prima facie* showing must contain the following elements:

(1) An explanation that clearly sets forth the reasoning used in concluding that the asserted utility for the claimed invention is not both specific and substantial nor well-established;

(2) Support for factual findings relied upon in reaching this conclusion; and

(3) An evaluation of all relevant evidence of record, including utilities taught in the closest prior art.

(b) Where the asserted specific and substantial utility is not credible, a *prima facie* showing of no specific and substantial credible utility must establish that it is more likely than not that a person skilled in the art would not consider credible any specific and substantial utility asserted by the applicant for the claimed invention.

The *prima facie* showing must contain the following elements:

(1) An explanation that clearly sets forth the reasoning used in concluding that the asserted specific and substantial utility is not credible;

(2) Support for factual findings relied upon in reaching this conclusion; and

(3) An evaluation of all relevant evidence of record, including utilities taught in the closest prior art.

(c) Where no specific and substantial utility is disclosed or is well-established, a *prima facie* showing of no specific and substantial utility need only establish that applicant has not asserted a utility and that, on the record before the examiner, there is no known well-established utility.

4. A rejection based on lack of utility should not be maintained if an asserted utility for the claimed invention would be considered specific, substantial, and credible by a person of ordinary skill in the art in view of all evidence of record.

Office personnel are reminded that they must treat as true a statement of fact made by an applicant in relation to

an asserted utility, unless countervailing evidence can be provided that shows that one of ordinary skill in the art would have a legitimate basis to doubt the credibility of such a statement. Similarly, Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered.

Once a *prima facie* showing of no specific and substantial credible utility has been properly established, the applicant bears the burden of rebutting it. The applicant can do this by amending the claims, by providing reasoning or arguments, or by providing evidence in the form of a declaration under 37 CFR 1.132 or a patent or a printed publication that rebuts the basis or logic of the *prima facie* showing. If the applicant responds to the *prima facie* rejection, the Office personnel should review the original disclosure, any evidence relied upon in establishing the *prima facie* showing, any claim amendments, and any new reasoning or evidence provided by the applicant in support of an asserted specific and substantial credible utility. It is essential for Office personnel to recognize, fully consider and respond to each substantive element of any response to a rejection based on lack of utility. Only where the totality of the record continues to show that the asserted utility is not specific, substantial, and credible should a rejection based on lack of utility be maintained.

If the applicant satisfactorily rebuts a *prima facie* rejection based on lack of utility under § 101, withdraw the § 101 rejection and the corresponding rejection imposed under § 112, first paragraph.

Dated: December 29, 2000.

Q. Todd Dickinson,

Under Secretary of Commerce for Intellectual Property and Director of the United States Patent and Trademark Office.

[FR Doc. 01–322 Filed 1–4–01; 8:45 am]

BILLING CODE 3510–16–U

DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

[Docket No. 991027288–0264–02]

RIN 0651–AB10

Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1, "Written Description" Requirement

AGENCY: United States Patent and Trademark Office, Commerce.

ACTION: Notice.

SUMMARY: These Guidelines will be used by USPTO personnel in their review of patent applications for compliance with the "written description" requirement of 35 U.S.C. 112, ¶ 1. These Guidelines supersede the "Revised Interim Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 'Written Description' Requirement" that were published in the **Federal Register** at 64 FR 71427, Dec. 21, 1999, and in the Official Gazette at 1231 O.G. 123, Feb. 29, 2000. These Guidelines reflect the current understanding of the USPTO regarding the written description requirement of 35 U.S.C. 112, ¶ 1, and are applicable to all technologies.

DATES: The Guidelines are effective as of January 5, 2001.

FOR FURTHER INFORMATION CONTACT: Stephen Walsh by telephone at (703) 305–9035, by facsimile at (703) 305–9373, by mail to his attention addressed to United States Patent and Trademark Office, Box 8, Washington, DC 20231, or by electronic mail at "stephen.walsh@uspto.gov"; or Linda Therkorn by telephone at (703) 305–8800, by facsimile at (703) 305–8825, by mail addressed to Box Comments, Commissioner for Patents, Washington, DC 20231, or by electronic mail at "linda.therkorn@uspto.gov."

SUPPLEMENTARY INFORMATION: As of the publication date of this notice, these Guidelines will be used by USPTO personnel in their review of patent applications for compliance with the "written description" requirement of 35 U.S.C. 112, ¶ 1. Because these Guidelines only govern internal practices, they are exempt from notice and comment rulemaking under 5 U.S.C. 553(b)(A).

Discussion of Public Comments

Comments were received from 48 individuals and 18 organizations in response to the request for comments on the "Revised Interim Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 'Written Description' Requirement" published in the **Federal Register** at 64 FR 71427, Dec. 21, 1999, and in the Official Gazette at 1231 O.G. 123, Feb. 29, 2000. The written comments have been carefully considered.

Overview of Comments

The majority of comments favored issuance of final written description guidelines with minor revisions. Comments pertaining to the written description guidelines are addressed in detail below. A few comments addressed particular concerns with respect to the associated examiner training materials that are available for public inspection at the USPTO web site (www.uspto.gov). Such comments will be taken under advisement in the revision of the training materials; consequently, these comments are not specifically addressed below as they do not impact the content of the Guidelines. Several comments raised issues pertaining to the patentability of ESTs, genes, or genomic inventions with respect to subject matter eligibility (35 U.S.C. 101), novelty (35 U.S.C. 102), or obviousness (35 U.S.C. 103). As these comments do not pertain to the written description requirement under 35 U.S.C. 112, they have not been addressed. However, the aforementioned comments are fully addressed in the "Discussion of Public Comments" in the "Utility Examination Guidelines" Final Notice, which will be published at or about the same time as the present Guidelines.

Responses to Specific Comments

(1) *Comment:* One comment stated that the Guidelines instruct the patent examiner to determine the correspondence between what applicant has described as the essential identifying characteristic features of the invention and what applicant has claimed, and that such analysis will lead to error. According to the comment, the examiner may decide what applicant should have claimed and reject the claim for failure to claim what the examiner considers to be the invention. Another comment suggested that the Guidelines should clarify what is meant by "essential features of the invention." Another comment suggested that what applicant has identified as the "essential distinguishing characteristics" of the invention should be understood in terms of *Fiers* v. *Revel*, 984 F.2d 1164, 1169, 25 USPQ2d 1601, 1605 (Fed. Cir. 1993) ("Conception of a substance claimed *per se* without reference to a process requires conception of its structure, name,

an asserted utility, unless countervailing evidence can be provided that shows that one of ordinary skill in the art would have a legitimate basis to doubt the credibility of such a statement. Similarly, Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered.

Once a *prima facie* showing of no specific and substantial credible utility has been properly established, the applicant bears the burden of rebutting it. The applicant can do this by amending the claims, by providing reasoning or arguments, or by providing evidence in the form of a declaration under 37 CFR 1.132 or a patent or a printed publication that rebuts the basis or logic of the *prima facie* showing. If the applicant responds to the *prima facie* rejection, the Office personnel should review the original disclosure, any evidence relied upon in establishing the *prima facie* showing, any claim amendments, and any new reasoning or evidence provided by the applicant in support of an asserted specific and substantial credible utility. It is essential for Office personnel to recognize, fully consider and respond to each substantive element of any response to a rejection based on lack of utility. Only where the totality of the record continues to show that the asserted utility is not specific, substantial, and credible should a rejection based on lack of utility be maintained.

If the applicant satisfactorily rebuts a *prima facie* rejection based on lack of utility under § 101, withdraw the § 101 rejection and the corresponding rejection imposed under § 112, first paragraph.

Dated: December 29, 2000.

Q. Todd Dickinson,

Under Secretary of Commerce for Intellectual Property and Director of the United States Patent and Trademark Office.

[FR Doc. 01–322 Filed 1–4–01; 8:45 am]

BILLING CODE 3510–16–U

DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

[Docket No. 991027288–0264–02]

RIN 0651–AB10

Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1, "Written Description" Requirement

AGENCY: United States Patent and Trademark Office, Commerce.

ACTION: Notice.

SUMMARY: These Guidelines will be used by USPTO personnel in their review of patent applications for compliance with the "written description" requirement of 35 U.S.C. 112, ¶ 1. These Guidelines supersede the "Revised Interim Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 'Written Description' Requirement" that were published in the **Federal Register** at 64 FR 71427, Dec. 21, 1999, and in the Official Gazette at 1231 O.G. 123, Feb. 29, 2000. These Guidelines reflect the current understanding of the USPTO regarding the written description requirement of 35 U.S.C. 112, ¶ 1, and are applicable to all technologies.

DATES: The Guidelines are effective as of January 5, 2001.

FOR FURTHER INFORMATION CONTACT: Stephen Walsh by telephone at (703) 305–9035, by facsimile at (703) 305–9373, by mail to his attention addressed to United States Patent and Trademark Office, Box 8, Washington, DC 20231, or by electronic mail at "stephen.walsh@uspto.gov"; or Linda Therkorn by telephone at (703) 305–8800, by facsimile at (703) 305–8825, by mail addressed to Box Comments, Commissioner for Patents, Washington, DC 20231, or by electronic mail at "linda.therkorn@uspto.gov."

SUPPLEMENTARY INFORMATION: As of the publication date of this notice, these Guidelines will be used by USPTO personnel in their review of patent applications for compliance with the "written description" requirement of 35 U.S.C. 112, ¶ 1. Because these Guidelines only govern internal practices, they are exempt from notice and comment rulemaking under 5 U.S.C. 553(b)(A).

Discussion of Public Comments

Comments were received from 48 individuals and 18 organizations in response to the request for comments on the "Revised Interim Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 'Written Description' Requirement" published in the **Federal Register** at 64 FR 71427, Dec. 21, 1999, and in the Official Gazette at 1231 O.G. 123, Feb. 29, 2000. The written comments have been carefully considered.

Overview of Comments

The majority of comments favored issuance of final written description guidelines with minor revisions. Comments pertaining to the written description guidelines are addressed in detail below. A few comments addressed particular concerns with respect to the associated examiner training materials that are available for public inspection at the USPTO web site (www.uspto.gov). Such comments will be taken under advisement in the revision of the training materials; consequently, these comments are not specifically addressed below as they do not impact the content of the Guidelines. Several comments raised issues pertaining to the patentability of ESTs, genes, or genomic inventions with respect to subject matter eligibility (35 U.S.C. 101), novelty (35 U.S.C. 102), or obviousness (35 U.S.C. 103). As these comments do not pertain to the written description requirement under 35 U.S.C. 112, they have not been addressed. However, the aforementioned comments are fully addressed in the "Discussion of Public Comments" in the "Utility Examination Guidelines" Final Notice, which will be published at or about the same time as the present Guidelines.

Responses to Specific Comments

(1) *Comment:* One comment stated that the Guidelines instruct the patent examiner to determine the correspondence between what applicant has described as the essential identifying characteristic features of the invention and what applicant has claimed, and that such analysis will lead to error. According to the comment, the examiner may decide what applicant should have claimed and reject the claim for failure to claim what the examiner considers to be the invention. Another comment suggested that the Guidelines should clarify what is meant by "essential features of the invention." Another comment suggested that what applicant has identified as the "essential distinguishing characteristics" of the invention should be understood in terms of *Fiers* v. *Revel*, 984 F.2d 1164, 1169, 25 USPQ2d 1601, 1605 (Fed. Cir. 1993) ("Conception of a substance claimed *per se* without reference to a process requires conception of its structure, name,

Regulation of left-right patterning in mice by growth/differentiation factor-1

Christopher T. Rankin[1], Tracie Bunton[2], Ann M. Lawler[3] & Se-Jin Lee[1]

The transforming growth factor-β (TGF-β) superfamily encompasses a large group of structurally related polypeptides that are capable of regulating cell growth and differentiation in a wide range of embryonic and adult tissues[1]. Growth/differentiation factor-1 (Gdf-1, encoded by *Gdf1*) is a TGF-β family member of unknown function that was originally isolated from an early mouse embryo cDNA library[2] and is expressed specifically in the nervous system in late-stage embryos and adult mice[3]. Here we show that at early stages of mouse development, *Gdf1* is expressed initially throughout the embryo proper and then most prominently in the primitive node, ventral neural tube, and intermediate and lateral plate mesoderm. To examine its biological function, we generated a mouse line carrying a targeted mutation in *Gdf1*. *Gdf1*$^{-/-}$ mice exhibited a spectrum of defects related to left-right axis formation, including visceral *situs inversus*, right pulmonary isomerism and a range of cardiac anomalies. In most *Gdf1*$^{-/-}$ embryos, the expression of *Ebaf* (formerly *lefty-1*) in the left side of the floor plate and *Leftb* (formerly *lefty-2*), *nodal* and *Pitx2* in the left lateral plate mesoderm was absent, suggesting that *Gdf1* acts upstream of these genes either directly or indirectly to activate their expression. Our findings suggest that *Gdf1* acts early in the pathway of gene activation that leads to the establishment of left-right asymmetry.

Northern-blot analysis of whole embryo RNA using a *Gdf1* probe detected two developmentally regulated transcripts, a 1.4-kb transcript containing the *Gdf1* coding region, which was expressed at early embryonic stages, and a bi-cistronic 3.0-kb transcript, which was expressed in later embryonic stages and in the nervous system of adult mice[3] (Fig. 1*a*). To examine the distribution of the 1.4-kb *Gdf1* transcript, we performed whole-mount *in situ* hybridization experiments on early stage mouse embryos. At 7.5 days post-coitum (d.p.c.), we detected *Gdf1* mRNA uniformly throughout the embryo proper, but not in extra-embryonic structures (Fig. 1*b,c*). By 8.0–8.5 d.p.c., we saw *Gdf1* mRNA in many tissues including the crown of the primitive node, ventral neural ectoderm and paraxial, intermediate and lateral plate mesoderm (Fig. 1*d–g*). At all stages examined, the expression pattern of *Gdf1* appeared to be bilaterally symmetric with respect to the left-right axis.

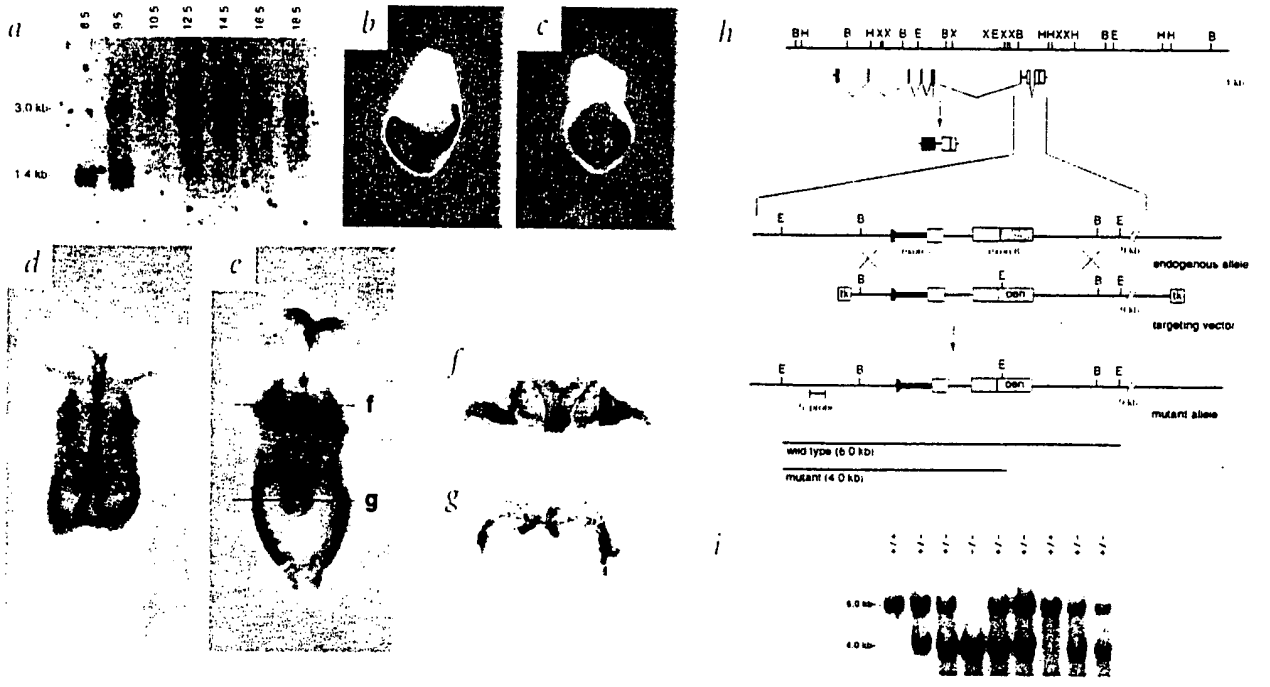


Fig. 1 *Gdf1* expression during early mouse development. ***a***, Northern-blot analysis of whole embryo RNA. Poly(A)$^+$-selected mRNAs prepared from CD-1 mouse embryos at the indicated d.p.c. were electrophoresed, blotted and probed with the entire *Gdf1* coding sequence. ***b–g***, Whole-mount *in situ* hybridization analysis of 7.5 d.p.c. (***b***, lateral view; ***c***, anterior view), 8.0 d.p.c. (***d***) and 8.5 d.p.c. (***e***) mouse embryos. Expression is symmetric at all stages examined. ***f,g***, Sections through a 8.5 d.p.c. embryo at the levels shown in (***e***). ***h***, Genomic map of the *Gdf1* locus and targeting strategy. Filled boxes represent the coding sequence for UOG-1 (ref. 3). Open and shaded boxes represent the coding sequences for the pro- and carboxy-terminal regions of Gdf-1, respectively. A probe derived from genomic sequence upstream of the targeting construct hybridizes to a 6-kb *Eco*RI fragment in wild-type *Gdf1* and a 4-kb *Eco*RI fragment in a homologously targeted *Gdf1* allele. ***i***, Genomic Southern blot of *Eco*RI-digested liver DNA prepared from newborn offspring of a mating of heterozygous mice.

[1]*Department of Molecular Biology and Genetics,* [2]*Division of Comparative Medicine and* [3]*Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. Correspondence should be addressed to S.-J.L. (e-mail: sjlee@jhmi.edu).*

intron 1 of human *F9*. Amplified vector sequence yields a PCR fragment of 743 bp. For each sample, a control reaction containing the sample to be tested spiked with vector plasmid (50 copies/μg DNA) was also run to establish that the sample did not inhibit the PCR reaction. For semen, 3 μg of DNA was analysed (1 μg in each of 3 separate reactions); for saliva and biopsied muscle, 1 μg; and for urine, serum and stool, DNA was extracted from a 1–2 ml volume and analysed. The sensitivity of the assay is 50 copies of vector sequence in 1 μg DNA.

Factor IX levels. We determined F.IX levels using an automated analyser (MDA, Organon-Teknika, or MLA-800, Medical Laboratory Automation). Plasma test samples were mixed with F.IX-deficient substrate (George King, Inc.), and results compared with the degree of correction obtained when dilutions of verify reference plasma were added to the same F.IX-deficient substrate. The reference curve was linear down to a lower limit of 0.3%.

The F.IX measurements reported here deserve comment, as the changes are small. Most clinical laboratories do not report a numerical value for clotting factor levels of <1%, but in preparation for this trial, the coagulation laboratories at CHOP and Stanford University Medical Center prepared detailed standard curves for F.IX, which were linear down to levels of ~0.3%. Most authorities would agree that an experienced clinical coagulation laboratory can distinguish between levels <1% and >1%. The values of >1% in patient A were actually repeated and verified by a third clinical laboratory. Thus it appears that these numbers represent an increase from the patient's true baseline, which was also verified to be <1% by three clinical laboratories before the beginning of the trial.

Acknowledgements

We thank A. Radu for assistance with immunohistochemical staining of muscle; M. Tanzer for assistance with coagulation assays; D. Leonard for assistance with PCR assays on human samples; the Nucleic Acid/Protein Research Core Facility at CHOP; R. Barth and M. King for assistance with ultrasound; and S.G. Madison for clinical support. This work was supported by National Institutes of Health Grants R01 HL53682 to M.A.K. and R01 HL53668, R01 HL61921, P50 HL54500 and a grant from the Hoxie Harrison-Smith Foundation to K.A.H. The work was also supported in part by NIH grant M01 RR00070 to the GCRC at Stanford University, by NIH grant M01 RR00240 to the GCRC at The Children's Hospital of Philadelphia and by Avigen.

Received 17 December 1999; accepted 27 January 2000.

1. Herzog, R.W. *et al.* Stable gene transfer and expression of human blood coagulation factor IX after intramuscular injection of recombinant adeno-associated virus. *Proc. Natl Acad. Sci. USA* **94**, 5804–5809 (1997).
2. Herzog, R. *et al.* Long-term correction of canine hemophilia B by gene transfer of blood coagulation factor IX mediated by adeno-associated viral vector. *Nature Med.* **5**, 56–63 (1999).
3. Ljung, R.C.R. Annotation. Can haemophilic arthropathy be prevented? *Br. J. Haematol.* **101**, 215–219 (1998).
4. Lofqvist, T., Nilsson, I.M., Berntorp, E. & Pettersson, H. Haemophilia prophylaxis in young patients—a long term follow up. *J. Intern. Med.* **241**, 395–400 (1997).
5. Mannucci, P.M. & Weitz, J.I. The hemophilias: progress and problems. *Semin. Hematol.* **36**, 104–117 (1999).
6. Parks, W.P., Boucher, D.W., Melnick, J.L., Taber, L.H. & Yow, M.D. Seroepidemiological and ecological studies of the adenovirus-associated satellite viruses. *Infect. Immun.* **2**, 716–722 (1970).
7. Summerford, C. & Samulski, R.J. Membrane-associated heparan sulfate proteoglycan is a receptor for adeno-associated virus type 2 virions. *J. Virol.* **72**, 1438–1445 (1998).
8. Qing, K. *et al.* Human fibroblast growth growth factor receptor 1 is a co-receptor for infection by adeno-associated virus 2. *Nature Med.* **5**, 71–77 (1999).
9. Cheung, W.F. *et al.* Identification of the endothelial cell binding site for factor IX. *Proc. Natl Acad. Sci. USA* **93**, 11068–11073 (1996).
10. Matsushita, T. *et al.* Adeno-associated virus vectors can be efficiently produced without helper virus. *Gene Ther.* **5**, 938–945 (1998).
11. Burton, M. *et al.* Coexpression of factor VIII heavy and light chain adeno-associated viral vectors produces biologically active protein. *Proc. Natl Acad. Sci. USA* **96**, 12725–12730 (1999).
12. Yoshitake, S., Schach, B.G., Foster, D.C., Davie, E.W. & Kuracki, K. Nucleotide sequence of the gene for human factor IX (antihemophilic factor B). *Biochemistry* **24**, 3736–3750 (1985).
13. Kurachi, S., Hitomi, Y., Midori, F. & Kurachi, K. Role of intron I in expression of the human factor IX gene. *J. Biol. Chem.* **270**, 5276–5281 (1995).
14. Manno, C.S., Larson, P.J., Cohen, A.R. & Flake, A.W. Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines). Appendix M. Points to consider in the design and submission of protocols for the transfer of recombinant DNA molecules into one or more human subjects. (NIH Recombinant DNA Advisory Committee, Washington DC, 1999).
15. Walter, J., You, Q., Hagstrom, J.N., Sands, M. & High, K.A. Successful expression of human factor IX following repeat administration of adenoviral vector in mice. *Proc. Natl Acad. Sci. USA* **93**, 3056–3061 (1996).
16. Miller, J.H. *Experiments in Molecular Genetics* 352–355 (Cold Spring Harbor Press, Cold Spring Harbor, New York, 1972).

Table 1 • Defects in *Gdf1*$^{-/-}$ newborn mice

		A	B	C	D	E	F	G	H	I
Heart malformation		✓	✓	✓	✓	✓	✓	✓	✓	✓
Right pulmonary isomerism		✓	✓	✓	✓	✓	✓	✓	✓	✓
GI tract, spleen and pancreas	normal	✓	✓	✓	✓	✓	–	–	–	–
	reversed	–	–	–	–	–	✓	✓	✓	✓
Liver	normal	✓	✓	–	–	–	–	–	–	–
	reversed	–	–	–	–	–	✓	✓	–	–
	symmetric	–	–	✓	✓	✓	–	–	✓	✓
Kidneys	normal	✓	–	✓	–	–	–	–	–	–
	reversed	–	–	–	✓	–	✓	–	✓	–
	symmetric	–	✓	–	–	✓	–	✓	–	✓
Total		10	2	2	2	1	9	1	4	3

Newborn *Gdf1*$^{-/-}$ mice were classified into groups on the basis of their combinations of anatomical *situs* defects.

To investigate the biological function of *Gdf1*, we generated mice in which the entire region encoding the mature Gdf-1 peptide was deleted by gene targeting (Fig. 1*h*). Among 481 offspring examined from *Gdf1*$^{+/-}$ intercrosses, only one homozygous mutant survived to adulthood. *Gdf1*$^{-/-}$ embryos appeared to be viable up to approximately 14.5 d.p.c. Only two-thirds of *Gdf1*$^{-/-}$ embryos survived until birth, however, and nearly all of these died within the first 48 hours after birth. The cause of death was likely to be related to the presence of extensive cardiac defects, although we have not ruled out the possibility that other abnormalities in these mutants might also have contributed to their severely reduced viability.

Homozygous mutant mice exhibited a complex spectrum of abnormalities related to improper establishment of left-right asymmetry (Table 1 and Fig. 2). The most obvious manifestations were the placement of the abdominal organs, which appeared to be randomized in *Gdf1*$^{-/-}$ mutants with respect to the left-right axis. Visceral *situs inversus* was most readily apparent with respect to the stomach, which is normally positioned on the left side (Fig. 2*a–c*). In 50% of *Gdf1*$^{-/-}$ mutants the stomach was positioned on the right side. In *Gdf1*$^{-/-}$ mice with right-sided stomachs, the direction of rotation of the small and large intestines was also reversed (Fig. 2*d,e*, and data not shown). Hence, the gastrointestinal tract appeared to have a mirror-image configuration relative to the left-right axis in approximately 50% of *Gdf1*$^{-/-}$ mice.

Abnormalities in left-right axis formation were evident in other abdominal organs as well. For example, the positions of the pancreas and spleen, which are normally left-sided (Fig. 2*b*), paralleled that of the stomach in *Gdf1*$^{-/-}$ mutants (Fig. 2*c*). The defects in the development of these organs, however, were more complex, as many *Gdf1*$^{-/-}$ mutants had an annular pancreas, and all had severely malformed spleens. Mutation of *Gdf1* also altered the relative positioning of the kidneys and adrenal glands, which are normally displaced caudally on the left side (Fig. 2*f*). In *Gdf1*$^{-/-}$ mice, the left kidney and adrenal were positioned either more cranially than the right kidney and adrenal (Fig. 2*g*) or at the same rostral-caudal level. Similarly, the normal asymmetric arrangement of the liver was also disrupted in *Gdf1*$^{-/-}$ mice. In wild-type mice, the liver consists of a large left lateral lobe, left and right medial lobes, and three smaller lobes on the right side. In *Gdf1*$^{-/-}$ mice, the liver lobes appeared to be reversed with respect to the left-right axis or bilaterally symmetric, with two equally sized lateral lobes beneath a fused medial lobe.

We also saw aberrant left-right patterning of the heart and lungs in homozygous mutants, but the nature of the defects in the thoracic organs appeared to be independent of whether or not the abdominal organs exhibited *situs inversus*. Wild-type mice have five lung lobes, a single large lobe on the left side and four smaller lobes on the right side (Fig. 2*h*). In contrast, *Gdf1*$^{-/-}$ mice had eight lung lobes that were symmetrically distributed with respect to the left-right axis (Fig. 2*i*). The duplication of the right-sided pattern, or right pulmonary isomerism, was observed in all homozygous mutants. The heart defects in *Gdf1*$^{-/-}$ mice were much more complex and variable. In wild-type mice, the apex of the heart points towards the left side of the animal (Fig. 3*d–f*). In *Gdf1*$^{-/-}$ mice, however, the position of the apex was randomized (Fig. 3*g–l*). All mutant hearts also showed abnormal positioning of the great vessels (Fig. 3*a–c*). The pulmonary artery (blue) normally exits the heart ventrally and to the right of the aorta (yellow). In contrast, the pulmonary artery in *Gdf1*$^{-/-}$ mice was positioned more dorsally than the aorta,

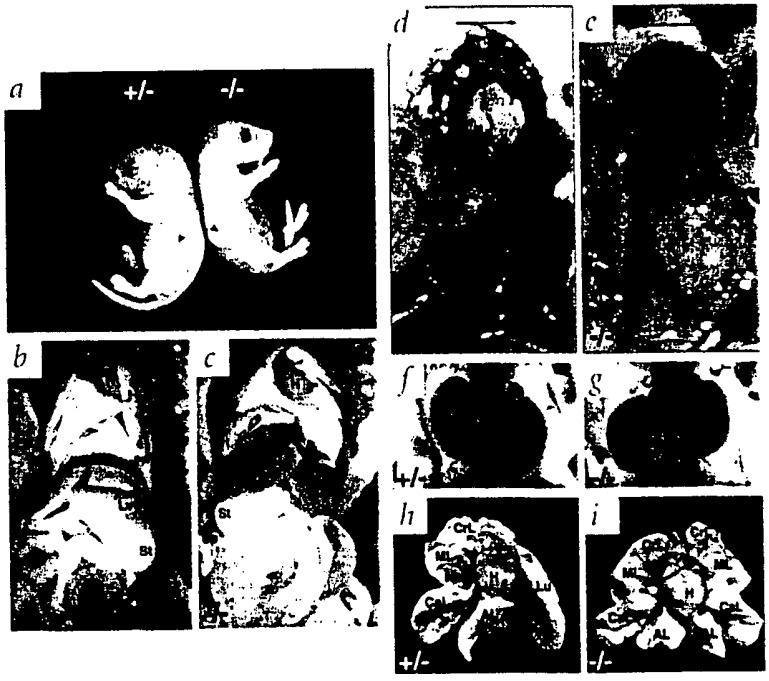


Fig. 2 Analysis of *situs* defects in *Gdf1*$^{-/-}$ mice. **a**, *Gdf1*$^{+/-}$ and *Gdf1*$^{-/-}$ newborn mice with stomachs (arrowheads) on the left and right sides, respectively. Ventral views of tissues from newborn *Gdf1*$^{+/-}$ (*b,d,f,h*) and *Gdf1*$^{-/-}$ (*c,e,g,i*) mice are shown. **b,c**, Reversal of the orientation of the abdominal organs in *Gdf1*$^{-/-}$ mice. Note also the streak-like appearance of the spleen and the abnormally shaped medial lobe of the liver. **d,e**, Reversal of the direction of rotation of the colon in *Gdf1*$^{-/-}$ mice. **f,g**, Reversal of the rostral-caudal arrangement of the kidneys in *Gdf1*$^{-/-}$ mice. **h,i**, Right pulmonary isomerism in *Gdf1*$^{-/-}$ mice. H, heart; Lv, liver; St, stomach; Sp, spleen; AC, ascending colon; DC, descending colon; RK, right kidney; LK, left kidney; CrL, cranial lung lobe; ML, medial lung lobe; CaL, caudal lung lobe; AL, accessory lung lobe; LLu, left lung lobe.

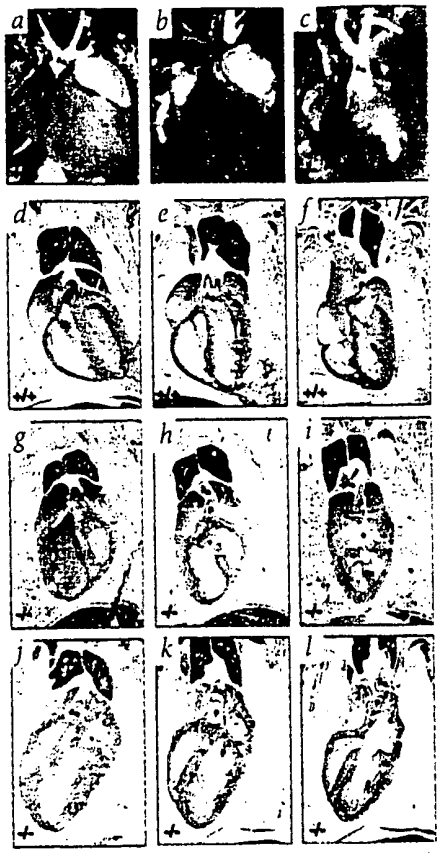


Fig. 3 Heart defects in *Gdf1*$^{-/-}$ mice. *a–c*, Dye injections of *Gdf1*$^{+/-}$ (**a**) and *Gdf1*$^{-/-}$ (**b,c**) mice. Yellow dye was injected into the ventricle positioned on the left side, and blue dye was injected into the ventricle on the right side. Note that the relative dorsal/ventral and or left/right relationships between the positions of the aorta and pulmonary artery are reversed in *Gdf1*$^{-/-}$ mice. Frontal sections of wild-type (**d,e,f**) and two *Gdf1*$^{-/-}$ (**g,h,i**, mesocardia; **j,k,l**, dextrocardia) hearts are shown. For each, three sections progressing from the ventral to the dorsal side are shown. In wild-type heart, the pulmonary artery exits the heart more ventrally than does the aorta. In *Gdf1*$^{-/-}$ mice, the aorta exits more ventrally. Ventricular septal defects (*h,k,l*, arrowheads) and atrial septal defects (*i*, asterisk) were also seen in *Gdf1*$^{-/-}$ mice.

although the left-right relationship of these arteries was randomized. In these mutants, the abnormal positioning of the aorta and pulmonary artery reflected a transposition of the great vessels. Histological examination of serial sections of five *Gdf1*$^{-/-}$ hearts revealed additional defects as well, including atrial and ventricular septal defects, common atrioventricular canal and persistent left vena cava (Fig. 3*g*, and data not shown).

To examine the relationship of *Gdf1* with other genes implicated in left-right determination, we carried out whole-mount *in situ* hybridization on *Gdf1*$^{-/-}$ embryos with probes directed against *Ebaf*, *Leftb* and *nodal*, encoding TGF-β family members, as well as *Pitx2*, encoding a transcription factor (Table 2). In wild-type embryos at the headfold stage (~4–6 somites), *Ebaf* is predominantly expressed on the left side of the presumptive floor plate, and *Leftb* is mainly expressed in the left lateral plate mesoderm[4] (LPM; Fig. 4*a*). Using a probe that detects both *Ebaf* and *Leftb* transcripts[5], we were unable to detect expression of these genes in most (6/7) headfold-stage *Gdf1*$^{-/-}$ embryos examined (Fig. 4*b*). Expression of *nodal* in headfold-stage wild-type embryos is normally seen in the node and left LPM (Fig. 4*c*; refs 6,7). Although the expression of *nodal* in the node remained unchanged, the expression of *nodal* in the LPM was absent in all mutant embryos examined (Fig. 4*d*). *Pitx2*, which is normally present in the left LPM of wild-type embryos[8,9] (Fig. 4*e*), was also downregulated in 8 of 11 *Gdf1*$^{-/-}$ embryos (Fig. 4*f*). These results suggest that *Gdf1* is necessary for the induction or maintenance of the asymmetric expression of *Ebaf*, *Leftb*, *nodal* and *Pitx2* in the early embryo.

Our findings indicate that *Gdf1* is essential for proper establishment of the left-right axis in mice. Although a number of other secreted proteins, including other members of the TGF-β superfamily, have been implicated as important regulators of left-right axis determination in vertebrates[10], Gdf-1 is unusual in that loss of Gdf-1 function leads to complete visceral *situs inversus* in a large percentage of mutant animals. In this regard, the *Gdf1*$^{-/-}$ phenotype more closely resembles that of mice carrying the *Dnahc11*^{iv}

Table 2 • Gene expression in *Gdf1*$^{-/-}$ embryos

			Floor plate				Lateral plate mesoderm				
Gene	Genotype	Somites	left	right	bilateral	absent	left	right	bilateral	absent	Total
Ebaf/Leftb	+/+	0–4	1	–	–	3	–	–	–	4	4
		4–6	5	–	–	1	6	–	–	–	6
		6–8	–	–	–	1	1	–	–	–	1
	+/–	0–4	5	–	–	4	–	–	–	9	9
		4–6	10	–	–	1	9	–	–	2	11
		6–8	2	–	–	6	8	–	–		8
	–/–	0–4	–	–	–	4				4	4
		4–6	1	–	–	6	1			6	7
		6–8	–	–	–	6	1			5	6

			Node		Lateral plate mesoderm				
			normal	absent	left	right	bilateral	absent	Total
nodal	+/+	0–4	6	–	–	–	–	6	6
		4–6	8	–	8	–	–		8
		6–10	3	1	–	–	–	4	4
	+/–	0–4	9	–	–	–	–	9	9
		4–6	12	–	12	–	–	–	12
		6–10	6	2	1	–	–	7	8
	–/–	0–4	6	–	–	–	–	6	6
		4–6	3	–	–	–	–	3	3
		6–10	7	2	1	–	–	8	9

			Lateral plate mesoderm				
			left	right	bilateral	absent	Total
Pitx2	+/+	6–10	5	–	–	–	5
	+/–	6–10	23	–	–	5	28
	–/–	6–10	3	–	–	8	11

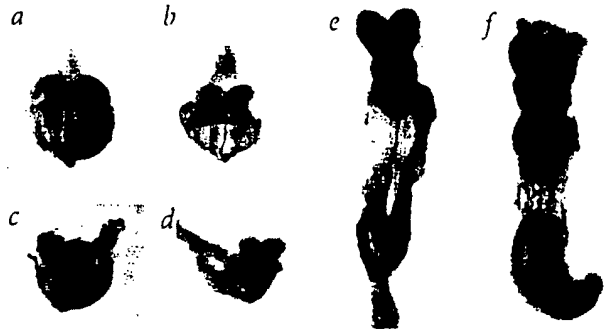


Fig. 4 Asymmetric gene expression in *Gdf1*$^{-/-}$ mice. Embryos in (*a–d*) are viewed from the anterior; (*e*) and (*f*) are seen from the ventral side. ***a***, Normal *Ebaf* and *Leftb* expression in the left LPM and floor plate. The expression of *Ebaf* and *Leftb* in the floor plate was verified by examining sections of these embryos. ***b***, Absence of visible *Ebaf* and *Leftb* expression in *Gdf1*$^{-/-}$ embryos. The midline staining in (*b*) represents background hybridization that did not correspond to the floor plate based on an analysis of sections of these embryos. ***c***, Normal expression of *nodal* in the left LPM (node staining is obscured by the LPM staining). ***d***, Absence of *nodal* expression in the LPM of *Gdf1*$^{-/-}$ embryos. Note the normal expression of *nodal* in the node. ***e***, Normal expression of *Pitx2* in the head mesenchyme and left LPM. ***f***, Absence of *Pitx2* expression in the LPM of *Gdf1*$^{-/-}$ embryos, whereas expression in the head mesenchyme remains normal.

allele[11,12] and that of humans with certain asplenia syndromes that are characterized by *situs inversus*[13,14]. *Gdf1* is also unusual in that its expression pattern during development appears to be symmetric with respect to the left-right axis. Although we cannot rule out the possibility that there is some asymmetric expression of *Gdf1* that is either very transient or below the level of detection in our experiments, our data suggest that the mechanism by which *Gdf1* influences left-right asymmetry must involve other molecules that are asymmetrically expressed, such as those responsible for generating biologically active Gdf-1 protein or for transducing the Gdf-1 signal. Alternatively, Gdf-1 protein may be synthesized in a symmetric pattern, but become asymmetrically distributed by some other mechanism. In this regard, it has been hypothesized that a leftward flow of fluid generated by cilia present in the node may be responsible for the directional movement of a morphogen produced by the node[15,16]. Given the intense expression of *Gdf1* in the node and the finding that *Gdf1* acts upstream of *Ebaf*, *Leftb*, *nodal* and *Pitx2*, Gdf-1 may be a possible candidate for this morphogen. An elucidation of the mechanism of action of Gdf-1 will require a careful examination of the distribution of the mature Gdf-1 protein and the identification of molecules directly involved in Gdf-1 signalling, including the Gdf-1 receptor.

Methods

In situ hybridization and northern-blot analysis. We carried out hybridizations using a probe corresponding to the entire *Gdf1* coding region[2]. We prepared the *Ebaf*, *Leftb* (ref. 5) and *nodal* (ref. 17) probes as described. The *Pitx2* probe was provided by M. Blum and M.R. Kuehn. Embryos were isolated from timed matings of CD-1 mice. We carried out northern-blot analysis using poly(A)-selected RNA (2 μg) as described[2]. Whole-mount *in situ* hybridization analysis was carried out as described[18,19], except that 20% heat-inactivated sheep serum was used for antibody blocking and incubation steps. Sections (10–12 μm) of stained embryos were prepared using a cryostat.

Construction and analysis of *Gdf1*-null mice. The structure of *Gdf1* was deduced from restriction mapping and partial sequencing of phage clones isolated from a mouse 129 Sv/J genomic library. Vectors for preparing the targeting construct were provided by P. Soriano and K. Thomas. RI embryonic stem cells (provided by A. Nagy, R. Nagy and W. Abramow-Newerly) were transfected with linearized targeting construct (50 μg) and selected with gancyclovir (2 μM) and G418 (200 μg/ml). Following electroporation of the targeting construct into embryonic stem cells, we identified homologous targeting by Southern-blot analysis in 4 of 90 clones resistant to both G418 and gancyclovir. Examination of the *Gdf1*-mutant phenotype was carried out using offspring on a C57Bl/6/129SV/J hybrid background from a chimaera derived from blastocyst injection of one of these clones. Genomic Southern blots were carried out as described[20]. We genotyped embryos by PCR using genomic DNA isolated from extra-embryonic membranes[21]. Primers for genotyping were as follows: *Gdf1* wild-type allele, 5′–GTTGCG GCTGGAGGCTGAGAG–3′ and 5′–CCCACTGGACCAACTTCTACC–3′; *Gdf1* targeted allele, 5′–CCACTGCAGCCTGTGGGCGC–3′ and 5′–GGAA GACAATAGCAGGCATGCTGG–3′.

For analysis of heart morphology, we performed latex dye injections as described[22]. Casting dyes (Connecticut Valley Biological Supply) were injected into the ventricles of the heart using a pulled capillary glass pipette. For histological analysis of *Gdf1*-mutant hearts, we killed newborn mice and infused them with Bouin's fixative through the trachea. The entire animal was then fixed by immersion in Bouin's fixative for at least 24 h. Mouse chests were step-sectioned (5 μM sections, 10 μM between each section) from the exit of the aorta or pulmonary artery to their respective branch points dorsally. For analysis, we stained sections with haematoxylin and eosin.

Acknowledgements

We thank P. Dunlap for assistance in maintaining mice and P. Wilcox for histotechnology support. C.T.R. was supported by a training grant from the NIH. This work was supported by a grants R01HD30740 and R01HD35887 from the NIH (to S.-J.L.).

Received 11 January; accepted 24 January 2000.

1. McPherron, A.C. & Lee, S.-J. The transforming growth factor β superfamily. in *Growth Factors and Cytokines in Health and Disease* (ed. LeRoith, D.) 357–393 (JAI, Greenwich, 1996).
2. Lee, S.-J. Identification of a novel member of the transforming growth factor-β superfamily. *Mol. Endocrinol.* **4**, 1034–1040 (1990).
3. Lee, S.-J. Expression of growth/differentiation factor 1 in the nervous system: conservation of a bicistronic structure. *Proc. Natl Acad. Sci. USA* **88**, 4250–4254 (1991).
4. Meno, C. *et al.* Two closely-related left-right asymmetrically expressed genes, *lefty-1* and *lefty-2*: their distinct expression domains, chromosomal linkage and direct neuralizing activity in *Xenopus* embryos. *Genes Cells* **2**, 513–524 (1997).
5. Meno, C. *et al.* Left-right asymmetric expression of the TGFβ-family member lefty in mouse embryos. *Nature* **381**, 151–155 (1996).
6. Collignon, J., Varlet, I. & Robertson, E.J. Relationship between asymmetric *nodal* expression and the direction of embryonic turning. *Nature* **381**, 155–158 (1996).
7. Lowe, L.A. *et al.* Conserved left-right asymmetry of nodal expression and alterations in murine *situs inversus*. *Nature* **381**, 158–161 (1996).
8. Yoshioka, H. *et al.* Pitx2, a bicoid-type homeobox gene is involved in a lefty-signaling pathway in determination of left-right asymmetry. *Cell* **94**, 299–305 (1998).
9. Piedra, M.E., Icardo, J.M., Albajar, M., Rodriguez-Rey, J.C. & Ros, M.A. Pitx2 participates in the late phase of the pathway controlling left-right asymmetry. *Cell* **94**, 319–324 (1998).
10. Ramsdell, A.F. & Yost, H.J. Molecular mechanisms of vertebrate left-right development. *Trends Genet.* **14**, 459–465 (1998).
11. Hummel, K.P. & Chapman, D.B. Visceral inversion and associated anomalies in the mouse. *J. Hered.* **50**, 9–13 (1959).
12. Layton, W.M. Random determination of a developmental process: reversal of normal visceral asymmetry in the mouse. *J. Hered.* **67**, 336–338 (1976).
13. Ivemark, B.I. Implications of agenesis of the spleen on the pathogenesis of conco-truncus anomalies in childhood. *Acta Paediatr. Scand.* **44**, 1–110 (1955).
14. Kosaki, K. & Casey, B. Genetics of human left-right axis malformations. *Semin. Cell Dev. Biol.* **9**, 89–99 (1998).
15. Nonaka, S. *et al.* Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. *Cell* **95**, 829–837 (1998).
16. Okada, Y. *et al.* Abnormal nodal flow precedes situs inversus in *iv* and *inv* mice. *Mol. Cell* **4**, 459–468 (1999).
17. Zhou, X., Sasaki, H., Lowe, L., Hogan, B.L.M. & Kuehn, M.R. *Nodal* is a novel TGFβ-like gene expressed in the mouse node during gastrulation. *Nature* **361**, 543–547 (1993).
18. Wilkinson, D.G. *In Situ Hybridization: A Practical Approach* 163 (Oxford University Press, New York, 1992).
19. Knecht, A.K., Good, P.J., Dawid, I.B. & Harland, R.M. Dorsal-ventral patterning and differentiation of noggin-induced neural tissue in the absence of mesoderm. *Development* **121**, 1927–1935 (1995).
20. McPherron, A.C., Lawler, A.M. & Lee, S.-J. Regulation of skeletal muscle mass in mice by a new TGF-β superfamily member. *Nature* **387**, 83–90 (1997).
21. Laird, P.W. *et al.* Simplified mammalian DNA isolation procedure. *Nucleic Acids Res.* **19**, 4293 (1991).
22. Oh, S.P. & Li, E. The signalling pathway mediated by the type IIB activin receptor controls axial patterning and lateral asymmetry in the mouse. *Genes Dev.* **11**, 1812–1826 (1997).

Familial dyserythropoietic anaemia and thrombocytopenia due to an inherited mutation in *GATA1*

Kim E. Nichols[1*], John D. Crispino[2*], Mortimer Poncz[3], James G. White[5], Stuart H. Orkin[2], John M. Maris[4] & Mitchell J. Weiss[3]

*These authors contributed equally to this work.

Haematopoietic development is regulated by nuclear protein complexes that coordinate lineage-specific patterns of gene expression. Targeted mutagenesis in embryonic stem cells and mice has revealed roles for the X-linked gene *Gata1* in erythrocyte and megakaryocyte differentiation[1–4]. GATA-1 is the founding member of a family of DNA-binding proteins that recognize the motif WGATAR through a conserved multifunctional domain consisting of two C4-type zinc fingers[5–8]. Here we describe a family with X-linked dyserythropoietic anaemia and thrombocytopenia due to a substitution of methionine for valine at amino acid 205 of GATA-1. This highly conserved valine is necessary for interaction of the amino-terminal zinc finger of GATA-1 with its essential cofactor, FOG-1 (for friend of GATA-1; refs 9–12). We show that the V205M mutation abrogates the interaction between Gata-1 and Fog-1, inhibiting the ability of Gata-1 to rescue erythroid differentiation in an erythroid cell line deficient for Gata-1 (G1E). Our findings underscore the importance of FOG-1:Gata-1 associations in both megakaryocyte and erythroid development, and suggest that other X-linked anaemias or thrombocytopenias may be caused by defects in *GATA1*.

A woman with mild chronic thrombocytopenia had two pregnancies complicated by severe fetal anaemia requiring *in utero* red blood cell transfusions. The offspring were male half-siblings who were anaemic and severely thrombocytopenic at birth and thereafter. Abnormalities were present in the erythrocyte and platelet lineages. Their peripheral blood (Fig. 1*a*) showed a paucity of platelets, and erythrocytes were abnormal in size and shape (poikilocytosis and anisocytosis). The bone marrow was hypercellular and contained an abundance of large, multinucleated, erythroid precursors (dyserythropoiesis; Fig. 1*c*) and numerous small, dysplastic megakaryocytes (Fig. 1*b*,*d*). Electron microscopy[13] also revealed dysplastic changes in megakaryocytes and platelets (Fig. 2). Both boys had cryptorchidism. There were three asymptomatic female siblings (Fig. 3*a*).

The haematopoietic defects in combination with a pedigree structure that was consistent with an X-linked disorder suggested the possibility of GATA-1 deficiency[3,4,14,15]. Therefore we analysed *GATA1* genomic DNA sequence from available family members at each of the five coding exons and approximately 800 bp of the promoter region (Fig. 3*a*). We identified a hemizygous G→A transition at nt 613 in both affected boys (II-1 and II-2). This mutation is predicted to convert valine to methionine at amino acid 205, a highly conserved residue within the GATA-1 protein. The boys inherited this mutation from their mother (I-2), who was heterozygous at nt 613. We did not detect the G613A mutation in peripheral blood leukocyte DNA from 50 normal females (data not shown).

The alteration of valine to methionine occurs in the N-terminal zinc finger, a region that is highly conserved between GATA-1 proteins of all species and in related proteins such as GATA-2–6 and the GATA-A (Pannier) protein of *Drosophila melanogaster* (Fig. 3*b*, and data not shown). *In vitro* studies have shown that the Gata-1 N-terminal zinc finger is essential for erythroid maturation, in large part due to its interaction with the zinc-finger protein Fog-1 (ref. 12). Valine 205 is one of several amino acids critical for direct association of Fog-1 with Gata-1 (Fig. 3*c*; refs 11,12,16). FOG-1 contains nine zinc fingers of multiple types, which presumably act as docking sites to link promoter-bound GATA-1 to other nuclear factors. Such multi-protein assemblies are likely to exert complex overall effects on gene expression, as FOG-1 can either activate or repress the activity of GATA-1, depending on cellular and promoter contexts[9,17].

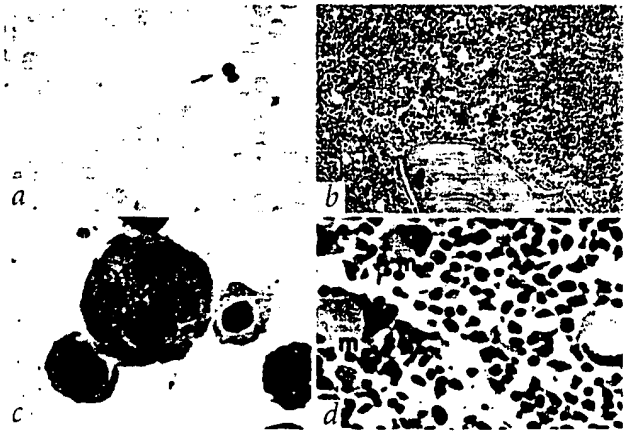


Fig. 1 Peripheral blood and bone marrow abnormalities in patients II-1 and II-2. ***a***, Peripheral blood smear. Note the severe deficiency of platelets and heterogeneity in red blood cell size (poikilocytosis) and shape (anisocytosis). An abnormal red blood cell with a bilobed nucleus is indicated (arrow). Original magnification ×50. ***b***, Bone marrow core biopsy. Megakaryocytes, distinguished by their pale pink cytoplasm, are abundant and abnormally small. Representative megakaryocytes are indicated (arrows). Original magnification ×10. ***c***, Bone marrow aspirate showing a cluster of developing erythroid precursors. Dyserythropoiesis is illustrated by the large, multinuclear erythroblast (centre). Original magnification ×100. ***d***, Bone marrow core biopsy. Megakaryocytes (m) are dysplastic with small ecentric nuclei. Original magnification ×50.

[1]*Massachusetts General Hospital Cancer Center, Charlestown, Massachusetts, USA.* [2]*Division of Hematology and Oncology, Children's Hospital and Howard Hughes Medical Institute, Harvard Medical School, Boston, Massachusetts, USA. Divisions of* [3]*Hematology and* [4]*Oncology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA.* [5]*Department of Laboratory Medicine, University of Minnesota, Minneapolis, Minnesota, USA. Correspondence should be addressed to M.J.W. (e-mail: weissmi@email.chop.edu).*

E

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION of:

LEE

Appln. No. 08/966,223 Group Art Unit: 1645

Filed: November 7, 1997 Examiner: M.P. Allen

FOR: GDF-1

* * *

RULE 132 DECLARATION

I, Ted Ebendal, declare and state as follows:

(1) I reside at Börjegatan 45 B, S-752 29 Uppsala, Sweden.

(2) I am a Professor and Chairman in the Department of Developmental Biology, Faculty of Medicine, Uppsala University since 1988. I hold a Ph.D. (Doctor of Philosophy) degree which was earned from Uppsala University, Sweden in 1976. A copy of my *curriculum vitae* is attached.

(3) I am an author of over 100 peer-reviewed publications in the field of neuronal growth factors and neurotrophic factors.

(4) Recombinant human GDF-1 (rhGDF-1) was provided by Michael Jarpe of Cambridge NeuroScience for use in the assays reported herein.

1E

(5) On information and belief, rhGDF-1 was produced as follows. The cDNA of human GDF-1 (amino acid residues 255 to 373) was cloned into pRSET (Invitrogen). The construct was designed to produce a fusion protein which adds 34 amino acid residues to the N-terminus of rhDGF-1 including six histidine residues. There is an enterokinase cleavage site between the N-terminal extension and the rhGDF-1 sequence to facilitate removal of the tag. However, this extension was not removed for the assays reported herein.

(6) On information and belief, the above-described expression construct was inserted into the *E. coli* cell line BL21(DE3)pLysS to induce rhGDF-1 expression. Expression was induced by the addition of IPTG and was allowed to proceed for 4 hours. rhGDF-1 was produced in inclusion bodies.

(7) On information and belief, the inclusion bodies containing rhGDF-1 were solubilized and folded in 6 M guanidine and 100 mM dithiothreitol. Reducing agent and denaturing agent was removed by reverse phase HPLC. The protein was dried down in a Speed Vac and resuspended in 8 M urea at 5 mg/ml protein concentration. The protein solution was then diluted 1/100 to a final concentration of 50 µg/ml in refolding buffer of 10 mM reduced glutathione, 1 mM oxidized glutathione, and 50 mM Tris buffer (pH 9.0). The rhGDF-1 protein was allowed to refold for 20 hours at 25°C.

(8) On information and belief, a sample of the refolded rhGDF-1 protein was then analyzed by reducing and non-reducing SDS-PAGE. The gel was stained with Coomassie and the proportion of dimer was determined by densitometry. The rhGDF-1 dimer was found to be approximately 20% of total protein. The rhGDF-1 preparation was stored at -80°C.

(9) The following assays were performed under my direction and the results were analyzed by me.

(10) The sample was assayed in a fibre outgrowth assay using sympathetic ganglia from embryonic day 9 chicken embryo explanted into a collagen gel. See Ebendal *et al.*, Journal of Neuroscience Research, vol. 40, pp. 276-284 for a description of the use of explanted ganglia in collagen gels. Neurotrophin-3 (NT-3) only weakly stimulates sympathetic fibre outgrowth in this assay (see panel d of Fig. 4 in Ernfors *et al.*, Proceedings of the National Academy of Science, U.S.A., vol. 87, pp. 5454-5458). Members of the TGF-beta superfamily of proteins potentiate the effects of NT-3 in this assay.

(11) The sample of GDF-1 was diluted 100-fold and then further diluted in culture medium with 1% fetal calf serum as a carrier. GDF-1 was assayed on sympathetic ganglia at a

TE

concentration of 2.5 to 250 ng/ml. The ganglia were examined after two days of incubation using darkfield microscopy. No fibre outgrowth was evoked by GDF-1 at any of these concentrations.

(12) Therefore, the potentiating effect of GDF-1 on neurotrophin activity could be assessed by comparing fibre outgrowth induced by NT-3 in the presence or the absence of GDF-1. Any increased fibre outgrowth caused by the combination of NT-3 and GDF-1 would be due to potentiation, instead of the effects of GDF-1 alone.

(13) The potentiating effect of GDF-1 in the sympathetic fibre outgrowth assay (Ernfors *et al.*, *id.*) was determined with human NT-3 (Austral Biologicals) at a concentration of 2 ng/ml and GDF-1 at concentrations between 0 to 250 ng/ml. Fibre outgrowth density was scored in a blinded fashion by two independent observers with culture dishes arranged in random order. Scores were recorded on a scale from 0 (no fibres) to 5 (very high density of fibres forming a circular halo around the explanted nervous tissue). The assay was repeated three times. The results below represent the mean of the scores given for each culture by each observer.

	GDF-1 concentration	Mean Score
Medium Only	0 ng/ml	0.0
NT-3 alone	0 ng/ml	1.7
GDF-1 alone	250 ng/ml	0.0
NT-3 + GDF-1	250 ng/ml	3.1
NT-3 + GDF-1	50 ng/ml	2.3
NT-3 + GDF-1	5 ng/ml	1.8

(14) The combination of GDF-1 at 250 ng/ml with NT-3 shows a significant potentiation effect in comparison to the response obtained with NT-3 alone (statistically significant difference at $P < 0.001$ using Mann-Whitney U test). There is also a clear trend of potentiation of NT-3 by GDF-1 at 50 ng/ml, although this difference is not statistically significant in the present format of the assay.

(15) The specific activity of GDF-1 in the assay shows a reasonable dose-response relationship between 50 to 250 ng/ml.

(16) In view of the above results, I conclude that GDF-1 has biological activity on neurons similar to members of the TGF-beta superfamily of proteins.

(17) I declare further that all statements made herein of my own knowledge are true and that all statements made on

information and belief are believed true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

x Ted Ebendal
Ted Ebendal

x April 9, 1998
Date

CURRICULUM VITAE for TED EBENDAL

Born: September 21, 1948. Stockholm, Sweden
Sex: Male
Marital status: Married, 1 child (born 1974)
Address: Börjegatan 45 B, S-752 29 UPPSALA, Sweden

Education/academic degrees:

1971 Bachelor of Science. Uppsala University, Sweden
1972 Master of Science, Uppsala University
1976 Doctor of Philosophy, Uppsala University
1977 Docent in Zoology, Uppsala University
1987 Professor, Faculty of Medicine, Uppsala University

Professional Experience:

1972-76	Teaching Instructor in Zoology, Uppsala University
1977	Assistant Professor in Zoology, Uppsala University
1977	Visiting scientist at Strangeways Research Laboratory, Cambridge, England
1977-81	Docent (Associate Professor) appointment in Zoology, Uppsala University
1981-87	Research Associate Professor in Neurobiology at the Swedish Natural Science Research Council
1988-	Full Professor of Developmental Biology, Faculty of Medicine, Uppsala University

Scientific Awards: King Oscar Prize, Uppsala University (1982). Erik K. Fernström's prize to especially promising young scientists (1991).

Invited Oral Presentations have been given at about 80 international meetings.

Current research interests: Molecular and developmental neuroscience, development and repair mechanisms in the nervous system, neuronal growth factors and their receptors including NGF, NT-3, GDNF and BMPs. Homologous recombination in transgenic mice using embryonic stem cells.

Administrative and scientific duties at the Faculty of Medicine, Uppsala University
Chairman at the Dept of Developmental Biology (1988-). Member of the Medical Faculty Board (1988-93). Member of various scientific priority committees etc (1988-), Vice Chairman of Neuroscience Center at Uppsala University, (1989-1995). Chairman Uppsala University Animal Research Board (1997-)
Pre-doctoral advisor: Supervised 11 PhD students of which 8 have finished their PhD thesis and three are on the way.
Post-doctoral advisor for: Wilma Friedman (USA, 1986-88), Reg Williams (Australia, 1991-94).

Organization of scientific meetings, courses etc: Organized EMBO and BMC Summer School courses for graduate students in Uppsala and at the Karolinska Institute and participated in the organization committees for international meetings in Sweden and abroad (ISDN Biennial Meeting in Tampere 1996, 5th NGF Meeting 1998).
Referee assignments etc: Exp. Cell Res., Exp. Brain Res.
Associate editor: J. Neurosci. Res., Int. J. Dev. Neurosci, Neuron, Alzheinmer's Disease.
Reviewer of applications for grants and research positions: Uppsala University, Karolinska Institute, Stockholm University, Umeå University. MRC Sweden, NSF USA and various Swedish Universities.
Member of scientific committees: The Swedish Foundation for Brain Research (1995-), The International Human Frontier Science Program (Brain Functions 1996-), The Royal Swedish Academy of Sciences National Committe on Biology (1997-).

Major funding (Principal Investigator): Swedish NFR (SEK 900,000/yr for 1997 to 1999).
Publications: Over 300 scientific papers, reviews and reports in the area of developmental neuroscience

SELECTED LIST OF PAPERS PUBLISHED BY TED EBENDAL

1 Ebendal, T. & Hedlund, K.-O. 1974. Histology of the chick embryo trigeminal ganglion and initial effects of its cultivation with and without nerve growth factor. Zoon 2: 25-35.

3 Ebendal, T. 1974. Scanning electron microscopy of the chick embryo nerve fibres and heart fibroblasts on collagen substrata *in vitro*. Zoon 2: 99-104.

4 Ebendal, T. & Hedlund, K.-O. 1975. Effects on nerve growth factor on the chick embryo trigeminal ganglion in culture. Zoon 3: 33-47.

5 Ebendal, T. 1975. Effects of nerve growth factor on the synthesis of nucleic acids and proteins in cultured chick embyo trigeminal ganglia. Zoon 3: 159-167.

6 Ebendal, T. & Jacobson, C.-O. Human glial cells stimulating outgrowth of axons in cultured chick embryo ganglia. Zoon 3: 169-172.

7 Ebendal, T. 1976. The relative roles of contact inhibition and contact guidance in orientation of axons extending on aligned collagen fibrils *in vitro*. Exp. Cell Res. 98: 159-169.

12 Ebendal, T. 1977. Extracellular matrix fibrils and cell contacts in the chick embryo: Possible roles in orientation of cell migration and axon extension. Cell Tissue Res. 175: 439-458.

13 Ebendal, T. 1976. Migratory mesoblast cells in the young chick embryo examined by scanning electron microscopy. Zoon 4: 101-108.

14 Ebendal, T. & Jacobson, C.-O. 1977. Tissue explants affecting extension and orientation of axons in cultured chick embryo ganglia. Exp. Cell Res. 105: 379-387.

16 Ebendal, T. & Jacobson, C.-O. 1977. Tests of possible role of NGF in neurite outgrowth stimulation by glial cells and heart explants in culture. Brain Res. 131: 373-378.

17 Ebendal, T. 1977. A cover glass dish for cell cultures intended for observation with inverted microscopes. Zoon 5: 73-75.

18 Ebendal, T. & Heath, J.P. 1977. Self-contact inhibition of movement in cultured chick heart fibroblasts. Exp. Cell Res. 110: 469-473.

19 Schultzberg, M., Ebendal, T., Hökfelt, T., Nilsson & Pfenninger, K. 1978. Substance P-like immunoreactivity in cultured spinal ganglia from chick embryos. J. Neurocytol. 7: 107-117.

20 Dunn, G. A. & Ebendal, T. 1978. Contact guidance on oriented collagen gels. Exp. Cell Res. 111: 475-479.

22 Dunn, G. A. & Ebendal, T. 1978. Some aspects on contact guidance. In: Formshaping Movements in Neurogenesis (ed. C.-O. Jacobson and T.Ebendal), Zoon 6: 217-223. Almqvist & Wiksell International, Stockholm.

23 Hedlund, K.-O. & Ebendal, T. 1978. Different ganglia from the chick embryo in

studies on neuron development in culture. In: Formshaping Movements in Neurogenesis (ed. C.-O. Jacobson and T. Ebendal), Zoon 6: 217-223. Almqvist & Wiksell International, Stockholm.

24 Ebendal, T., Jordell-Kylberg, A. & Söderström, S. 1978. Stimulation by tissue explants on nerve fibre outgrowth in culture. In: Formshaping Movements in Neurogenesis (ed. C.-O. Jacobson and T. Ebendal), Zoon 6: 235-243. Almqvist & Wiksell International, Stockholm.

27 Ebendal, T. 1979. Stage-dependent stimulation of neurite outgrowth exerted by nerve growth factor and chick heart in cultured embryonic ganglia. Dev. Biol. 72: 276-290.

28 Ebendal, T., Belew, M., Jacobson, C.-O. & Porath, J. 1979. Neurite outgrowth elicited by embryonic chick heart: partial purification of the active factor. Neurosci. Lett. 14: 91-95.

29 Olson, L., Ebendal, T. & Seiger, Å. 1979. NGF and anti-NGF: Evidence against effects on fiber growth in locus coeruleus from cultures of perinatal CNS tissues. Dev. Neurosci. 2: 160-176.

32 Löfberg, J. & Ebendal, T. 1979. Substrate topography and shape of motile cells. Experientia 36: 508-510.

33 Olson, L., Seiger, Å., Ebendal, T. & Hoffer, B. 1980. Comparisons of nerve fiber growth from three major catecholamine producing cell systems: adrenal medulla, superior cervical ganglion and locus coeruleus. In: Histochemistry and Cell Biology of Autonomic Neurons, SIF cells and Paraneurons (ed. O. Eränkö et al.), pp. 27-34. Raven Press, N.Y.

34 Jacobson, C.-O., Ebendal, T., Hedlund, K.-O. & Norrgren, G. 1981. Factors stimulating neurite outgrowth in chick embryo ganglia. In: Control Mechanisms in Animal Cells - Specific Growth Factors (ed. L. Jimenez de Azua et al.), pp. 325-332. Raven Press, N.Y.

35 Hedlund, K.-O. & Ebendal, T. 1980. The chick embryo nodose ganglion: effects of nerve growth factor in culture. J. Neurocytol. 9: 665-682.

36 Ebendal, T., Olson, L., Seiger, Å. & Hedlund, K.-O. 1980. Nerve growth factors in the rat iris. Nature (London) 286: 25-28.

37 Norrgren, G., Ebendal, T., Belew, M., Jacobson, C.-O. & Porath, J. 1980. Release of nerve growth factor by human glial cells in culture. Exp. Cell Res. 130: 31-39.

41 Ebendal, T. 1981. Control of neurite extension by embryonic heart explants. J. Embryol. Exp. Morphol. 61: 289-301.

42 Ebendal, T. 1982. Orientational behaviour of extending neurites. In: Cell Behaviour (ed. R. Bellairs, A.S.G. Curtis & G. Dunn) pp. 281-297. Cambridge University Press.

44 Olson, L., Björklund, H., Ebendal, T., Hedlund, K.-O. & Hoffer, B. 1981. Factors regulating growth of catecholamine-containing nerves, as revealed by transplantation experiments. Ciba Found. Symp. 83: 213-231.

49 Richardson, P.M. & Ebendal, T. 1982. Nerve growth activities in rat peripheral

nerve. Brain Res. 246: 57-64.

50 Carri, N.G. & Ebendal, T. 1983. Organotypic cultures of neural retina: neurite outgrowth stimulated by brain extracts. Dev. Brain Res. 6: 219-229.

52 Ebendal, T., Hedlund, K.-O., & Norrgren, G. 1982. Nerve growth factors in chick tissues. J. Neurosci. Res. 8: 153-164.

53 Ebendal, T., Norrgren, G. & Hedlund, K.-O. 1983. Nerve growth-promoting activity in the chick embryo: Quantitative aspects. Med. Biol. 61: 65-72.

56 Ebendal, T. 1984. Nerve growth-promoting activities in embryonic and adult tissues. In: Organizing Principles of Neural Development (ed. S.C. Sharma), pp. 93-107, NATO ASI Series A: Vol. 78, Plenum Press, N. Y.

57 Norrgren, G., Ebendal,T., Gebb, C. & Wikström, H. 1984. The use of Cytodex 3 microcarriers and reduced-serum media for the production of nerve growth promoters from chicken heart cells. Devel. Biol. Stand. 55: 43-51.

58 Ebendal, T., Norrgren, G., Carri, N.G. & Belew, M. 1985. Nerve growth factors in peripheral tissues and CNS. In: Upper Motor Neuron Functions and Dysfunctions, Recent Achievements in Restorative Neurology. Vol. I (ed. Sir John Eccles & M.R. Dimitrijevic), pp. 311-320. Karger, Basel.

59 Ebendal, T., Olson, L., Seiger, Å. & Belew, M. 1984. Nerve growth factors in chick and rat tissues. In: Cellular and Molecular Biology of Neuronal Development (ed. I. B. Black) pp. 231-242. Plenum Press, New York.

60 Ebendal, T., Olson, L. & Seiger, Å. 1983. The level of nerve growth factor (NGF) as a function of innervation: A correlative radioimmunoassay and bioassay study of the rat iris. Exp. Cell Res. 148: 311-317.

70 Norrgren, G., Wikström, H. & Ebendal, T. 1984. Production of nerve growth promoting factor(s) from chick embryo heart cells. Use of Cytodex 3 microcarriers and serum-free media. Exp. Cell Res. 152: 427-435.

71 Ayer-LeLièvre, C.S., Ebendal, T., Olson, L. & Seiger, Å. 1983. Localization of nerve growth factor-like immunoreactivity in rat nervous tissue. Med. Biol. 61: 296-304.

73 Olson, L., Ebendal, T. & Seiger, Å. 1984. Intraocular grafting of cultured brain tissue: Growth, vascularizaion and neuron survival in locus coeruleus and cortex cerebri. Neurosci. Lett. 47: 139-144.

74 Ebendal, T. & Lundin, L.-G. 1984. Nerve growth factor in three neurologically deficient mouse mutants (*Swl*, *Sp* and *dt*^{J}). Neurosci. Lett. 50: 121-126.

75 Richardson, P.M., Ebendal, T. & Riopelle, R. 1985. Nerve growth and nerve growth factor within peripheral nervous tissue. In: Neural Grafting in the Mammalian CNS (ed. A. Björklund and U. Stenevi), pp. 319-327. Elsevier.

76 Olson, L., Strömberg, I. Herrera-Marschitz, Ungerstedt, U. & Ebendal, T. 1985. Adrenal medullary tissue grafted to the dopamine-denervated rat striatum: Histochemical and functional effects of additions of nerve growth factor. In: Neural Grafting in the Mammalian CNS. (ed. A. Björklund and U. Stenevi), pp. 505-518. Elsevier.

78 Ebendal, T., Lärkfors, L., Ayer-LeLievre, C., Seiger, Å. & Olson, L. 1985. New approaches to detect NGF-like activity in tissues. In: Hormones and Cell Regulation, Vol. 9 (ed. J.E. Dumont, B. Hamprecht & J. Nunez), pp. 361-376. Elsevier, Amsterdam.

79 Strömberg, I., Ebendal, T., Seiger, Å. & Olson, L. 1985. Nerve fiber production by intraocular adrenal medullary grafts: Stimulation by nerve growth factor or sympathetic denervation of the host iris. Cell Tissue Res. 241: 241-249.

80 Strömberg, I., Herrera-Marschitz, M., Ungerstedt, U., Ebendal, T. & Olson, L. 1985. Chronic implants of chromaffin tissue into the dopamine-denervated striatum. Effects of NGF on survival, fiber growth and rotational behavior. Exp. Brain Res. 60: 335-349.

82 Norrgren, G. & Ebendal, T. 1986. Nerve growth factor in medium conditioned by embryonic chicken heart cells. Int. J. Devl. Neurosci. 4: 41-49.

95 Carri, N.G. & Ebendal, T. 1986. Selected optic lobe extracts stimulating retinal neurite outgrowth. In: Progress in Developmental Biology, part A (ed. H.C. Slavkin). Alan R. Liss, Inc., N.Y., pp. 119-122.

96 Belew, M. & Ebendal, T. 1986. Chick embryo nerve growth factor. Fractionation and biological activity. Exp. Cell Res. 167: 550-558.

97 Carri, N.G. & Ebendal, T. 1986. A method for explantation of selected areas of the neural retina. Anat. Rec. 214: 226-229.

98 Whittemore, S.R., Ebendal, T., Lärkfors, L., Seiger, Å., Strömberg, I. & Persson, H. 1986. Developmental and regional expression of ß nerve growth factor messenger RNA and protein in the rat central nervous system. Proc. Natl. Acad. Sci. USA 83: 817-821.

99 Thompson, W.J., Bicker, G., Changeux, J.-P., Ebendal, T.L., Heisenberg, M., Henderson, C.E., Huttner, W., Kandel, E.R., Mallet, J.B., Stent, G.S., Thoenen, H. & Yaniv, M. 1987. Activity-dependent regulation of gene expression (Group report). In: The Neural and Molecular Bases of Learning (ed. J.-P. Changeux & M. Konishi). Dahlem Workshop Reports, Life Sciences 38: 13-29. John Wiley, Chichester.

100 Whittemore, S.R., Lärkfors, L., Ebendal, T., Ericsson, A., Holets, V.R. & Persson, H. 1987. Increased ß-nerve growth factor messenger RNA and protein in neonatal rat hippocampus following specific cholinergic lesions. J. Neurosci. 7: 244-251.

101 Carri, N.G. & Ebendal, T. 1987. Target-field specificity in the induction of retinal neurite outgrowth. Dev. Brain Res. 31: 83-90.

102 Lärkfors, L. & Ebendal, T. 1987. Highly sensitive immunoassays for ß-nerve growth factor. J. Immunol. Meth. 97: 41-47.

103 Ebendal, T., Larhammar, D. & Persson, H. 1986. Structure and expression of the chicken ß-nerve growth factor gene. EMBO J. 5: 1483-1487.

104 Dimberg, Y. & Ebendal, T. 1987. Effects of nerve growth factor on autonomic neurons in the chick embryo. A stereological study: Int. J. Devl. Neurosci. 5: 195-205.

105 Lärkfors, L., Strömberg, I., Ebendal, T. & Olson, L. 1987. Nerve growth factor protein level increases in the adult rat hippocampos after a specific cholinergic lesion. J. Neurosci. Res. 18: 525-531.

106 Olson, L., Ayer-LeLievre, C., Ebendal, T. & Seiger, Å. 1987. Nerve growth factor-like immunoreactivities in rodent salivary glands and testis. Cell Tissue Res. 248: 275-286.

107 Olson, L., Ayer-LeLievre, C., Ebendal, T. & Seiger, Å. 1987. NGF-like immunoreactivities in rodent salivary glands and testis. Cell Tissue Res. 248: 275-286.

108 Dimberg, Y., Hedlund, K.-O. & Ebendal, T. 1987. Effects of ß-nerve growth factor on sensory neurons in the chicken embryo. A stereological study. Int. J. Devl. Neurosci. 5: 207-213.

109 Ebendal, T. 1987. Comparative screening for ciliary neurotrophic activity in organs of the rat and chicken. J. Neurosci. Res. 17: 19-24.

110 Ebendal, T., Askmark, H. & Aquilonius, S.-M. 1989. Screening for neurotrophic disturbances in amyotrophic lateral sclerosis. Acta Neurol. Scand. 79: 188-193.

114 Aquilonius, S.-M., Askmark, H., Ebendal, T. & Gillberg, P.-G. 1987. Neuropharmacology of motor neuron disease. In: Cellular and Molecular Basis of Cholinergic Function. (ed. M. J. Dawdal and Hawthorne, J. N.), pp. 729-735. Wiley, Chichester.

116 Lärkfors, L., Ebendal, T., Whittemore, S.R., Persson, H., Hoffer, B. & Olson, L. 1987. Decreased level of nerve growth factor (NGF) and its messenger in the aged rat brain. Mol. Brain Res. 3: 55-60.

117 Ayer-LeLievre, C., Olson, L., Ebendal, T., Seiger, Å. & Persson, H. 1988. Expression of the ß-nerve growth factor gene in hippocampal neurons producing NGF mRNA in the brain by *in situ* hybridization. Science 240: 1339-1341.

119 Hallböök, F., Ebendal, T., & Persson, H. 1988. Production and characterization of biologically active recombinant beta nerve growth factor. Mol. Cell. Biol. 8: 452-456.

120 Ayer-LeLievre, C., Olson, L., Ebendal, T., Hallböök, F. & Persson, H. 1988. Nerve growth factor mRNA and protein in the testis and epididymis of mouse and rat. Proc. Natl. Acad. Sci. USA 85: 2628-2632.

121 Carri, N.G., Perris, R., Johansson, S. & Ebendal, T. 1988. Differential outgrowth of retinal neurites on purified extracellular matrix molecules. J. Neurosci. Res. 19: 428-439.

122 Ebendal, T. & Persson, H. 1988. Detection of nerve growth factor mRNA in the developing chicken embryo. Development 102: 101-106.

123 Ebendal, T & Persson, H. 1988. Developmental expression of nerve growth factor. In: Neural Development and Regeneration (ed. A. Gorio et al.). NATO ASI Series, Vol. H 22: 233-244. Springer, Berlin.

124 Whittemore, Scott R., Persson, H., Ebendal, T., Lärkfors, L., Larhammar, D. &

Ericsson, A. 1988. Structure and expression of ß-nerve growth factor in the rat central nervous system. In: Neural Development and Regeneration (ed. A. Gorio et al.). NATO ASI Series, Vol. H 22: 245-256. Springer, Berlin.

125 Strömberg, I, Hultgård-Nilsson, A., Hedin, U. & Ebendal, T. 1988. Fate of intraocular chromaffin cell suspensions: role of initial nerve growth factor support. Cell Tissue Res. 254: 487-497.

142 Dimberg, Y. & Ebendal, T. 1988. Effects on injecting antibodies to mouse nerve growth factor into the chick embryo. Int. J. Devl. Neurosci. 6: 513-523.

143 Ernfors, P., Hallböök, F., Ebendal, T., Schooter, E.M., Radeke, M.J., Misko, T.P. & Persson, H. 1988. Developmental and regional expression of ß-nerve growth factor receptor mRNA in the chick and rat. Neuron 1: 983-996.

147 Ayer-LeLievre, C., Ebendal, T., Olson, L., Seiger, Å. & Persson, H. 1989. Detection of nerve growth factor and its mRNA by separate and combined immunohistochemistry and *in situ* hybridization in mouse salivary glands. Histochem. J. 21: 1-7.

148 Lärkfors, L., Ebendal, T., Whittemore, S.R., Persson, H., Hoffer, B. & Olson, L. 1988. Developmental appearance of nerve growth factor in the rat brain: significant deficits in the aged forebrain. In: Transplantation into the Mammalian CNS (ed. D.M. Gash & J. R. Sladek, Jr.), Progr. Brain Res. 78: 27-31. Elsevier, Amsterdam.

149 Ebendal, T., Persson, H., Larhammar, D., Lundströmer, K. & Olson, L. 1989. Characterization of antibodies to synthetic nerve growth factor (NGF) and proNGF peptides. J. Neurosci. Res. 22: 223-240.

150 Nylén, P., Ebendal, T., Eriksdotter-Nilsson, M., Hansson, T., Henschen, A., Johnson, A.-C., Kronevi, T., Kvist, U., Sjöstrand, N.O., Höglund, G. & Olson, L. 1989. Testicular atrophy and loss of nerve growth factor-immunoreactive germ cell line in rats exposed to n-hexane and a protective effect of simultaneous exposure to toluene or xylene. Arch. Toxicol. 63: 296-307.

152 Eriksdotter-Nilsson, M., Skirboll, S.,Ebendal, T., Hersh, L., Grassi, J., Massoulié, J. & Olson, L. 1989. NGF treatment promotes development of basal forebrain tissue grafts in the anterior chamber of the eye. Exp. Brain Res. 74: 89-98.

153 Eriksdotter-Nilsson, M., Skirboll, S., Ebendal, T. & Olson, L. 1989. Nerve growth factor can influence growth of cortex cerebri and hippocampus: Evidence from intraocular grafts. Neuroscience 30: 755-766.

154 Strömberg, I. & Ebendal, T. 1989. Aged adrenal medullary tissue survives intraocular grafting, forms nerve fibers and responds to nerve growth factor. J. Neurosci. Res. 23: 162-171.

155 Ebendal, T. 1989. Use of collagen gels to bioassay nerve growth factor activity. In: Nerve growth factors (ed. R. A. Rush). IBRO Handbook series: Methods in the Neurosciences Vol. 12: 81-93. John Wiley, Chichester.

157 Hallböök, F., Persson, H., Barbany, G. & Ebendal, T. 1989. Developmental and regional expression of chicken neuroleukin (glucose-6-phosphate isomerase) messenger RNA. J. Neurosci. Res. 23: 142-151.

158 Olson, L., Ayer-Le Lievre, C., Bygdeman, M., Ebendal, T., Ernfors, P., Eriksdotter-Nilsson, M., Giacobini, M.B., Henschen, A., Hoffer, B., Mouton, P., Palmer, M., Persson, H., Sara, V., Seiger, Å., Strömberg, I. & Trok, K. 1989. Brain tissue transplantation and growth factors: Basic research and clinical applications. In: Bioinformatics. Information, Transduction and Processing Systems from Cell to Whole Body (O. Hatase and J. H. Wang, eds.), pp. 297-304, Elsevier, Amsterdam.

159 Carri. N.G. & Ebendal, T. 1989. Staining of developing neurites with Coomassie blue. Stain Tech. 64: 50-52.

160 Olson, L., Ayer-Lievre, C., Ebendal, T., Eriksdotter-Nilsson, M., Ernfors, P., Mouton, P., Persson, H. & Strömberg, I. 1989. NGF in CNS: Sites of synthesis and effects of novel ways to administer NGF on intrinsic cholinergic neurons and grafts of cholinergic neurons and their target areas. In: Neuronal Grafting and Alzheimer's Disease (F. Gage, A. Privat and Y. Christens, eds.), pp. 73-84. Springer, Berlin.

161 Ernfors, P., Ebendal, T., Olson, L., Mouton, P., Strömberg, I. & Persson, H. 1989. A cell line producing recombinant nerve growth factor evokes growth responses in intrinsic and grafted central cholinergic neurons. Proc. Natl. Acad. Sci. USA. 86: 4756-4760.

170 Ebendal, T. 1989. NGF in CNS: Experimental data and clinical implications. Progr. Growth Factor Res. 1: 143-159.

171 Ebendal, T. 1990. Developmental control of nerve growth factor expression in the nervous system. In: Regulation of Gene Expression in the Nervous System (A.-M. Giuffrida-Stella, J. de Vellis and J. Regino Perez-Polo, eds.). Neurology and Neurobiology, Vol. 59, pp. 19-30, Wiley-Liss, New York.

172 Persson, H., Ernfors, P., Friedman, W., Hallböök, F., Ayer-LeLievre, C., Ebendal, T.,Olson, L., Henschen, A., Mouton, P. & Strömberg, I. 1990. Expression of β-nerve growth factor and its receptor in the mammalian central nervous system. In: Brain Repair (ed. A. Björklund, A.J. Aguayo and D. Ottoson). Wenner-Gren Intern. Symp. Series Vol. 56, pp. 73-86. MacMillan Press, London.

173 Ebendal, T., Hallböök, F., Ibáñez, C., Persson, H. & Lärkfors, L. 1990. Activity and immunological properties of recombinant nerve growth factor (β-NGF). In: Brain Repair (A. Björklund, A. J. Aguayo and D. Ottoson, eds.). Wenner-Gren Intern. Symp. Series Vol. 56, pp. 57-71. MacMillan Press, London.

175 Olson, L., Ayer-LeLievre, C., Ebendal, T., Eriksdotter-Nilsson, M., Ernfors, P., Henschen, A., Hoffer, B., Giacobini, M., Mouton, P., Palmer, M., Persson, H., Sara, V., Strömberg, I. & Wetmore, C. 1990. Grafts, growth factors, and grafts that make growth factors. In: Neural Transplantation: From Molecular Bases to Clinical Application. Progr. Brain Res. 82: 55-65.

176 Strömberg, I., Ebendal, T., Olson, L. & Hoffer, B. 1990. Chromaffin grafts:

Survival and nerve fiber formation as a function of donor age, nerve growth factor and host sympathetic denervation. Progr. Brain Res. 82: 87-93.

177 Friedman, W., Lärkfors, L., Ayer-LeLievre, C., Ebendal, T., Olson, L. and Persson, H. 1990. Regulation of ß-nerve growth factor expression by inflammatory mediators in hippocampal cultures. J. Neurosci. Res. 27: 374-382.

179 Strömberg, I., Wetmore, C.J., Ebendal, T., Ernfors, P., Persson, H. & Olson, L. 1990. Rescue of basal forebrain cholinergic neurons after implantation of genetically modified cells producing recombinant NGF. J. Neurosci. Res. 25: 405-411.

180 Strömberg, I., Ebendal, T., Eriksdotter-Nilsson, M., Ernfors, P., Friedman, W., Persson, H., Wetmore, C, & Olson, L. 1990. Strategies to increase NGF levels and effect thereof on lesioned and grafted brain tissue. In: Brain Repair (A. Björklund, A.J. Aguayo and D. Ottoson, eds.). Wenner-Gren Intern. Symp. Series Vol. 56, pp. 87-98, Mac Millan Press, London.

181 Mohammed, A. K., Winblad, B., Ebendal, T. & Lärkfors, L. 1990. Environmental influence on behaviour and nerve growth factor in the brain. Brain Res. 528: 62-72.

185 Hallböök, F., Ayer-LeLievre, C., Ebendal, T & Persson, H. 1990. Expression of nerve growth factor receptor mRNA during early development of the chicken embryo - emphasis on cranial ganglia. Development 108: 693-704.

187 Ibáñez, C.F., Hallböök, F., Ebendal, T. & Persson, H. 1990. Structure-function studies of nerve growth factor: Functional importance of highly conserved amino acid residues. EMBO J. 9: 1477-1483.

191 Ernfors, P., Ibáñez, C. F., Ebendal, T., Olson, L. & Persson, H. 1990. Molecular cloning and neutrophic activities of a protein with structural similarities to nerve growth factor: Developmental and topographical expression in the brain. Proc. Natl. Acad. Sci. USA, 87: 5454-5458.

192 Olson, L., Eriksdotter-Nilsson, M., Ernfors, P., Henschen, A., Strömberg, I., Wetmore, C., Ebendal, T. & Persson, H. 1990. On the roles of neurotrophic factors in the CNS: Focus on the NGF/BDNF family. Third Conference, Institute of Developmental Neuroscience and Aging: "Plasticity and regeneration of the nervous system". (Abstr.).

193 Aquilonius, S-M., Askmark, H. Ebendal, T. & Gillberg, P.-G. 1992. No re-expression of high-affinity nerve growth factor binding sites in spinal motor neurons in amyotrophic lateral sclerosis. Eur. Neurol. 32: 216-218.

195 Westermark, K., Nilsson, M., Ebendal, T. & Westermark, B. 1991. Thyrocyte migration and histiotypic follicle regeneration are promoted by EGF in primary culture of thyroid follicles in collagen gel. Endocrinology 129: 2180-2186.

197 Olson, L., Ayer-LeLievre, C., Björklund, H., Ebendal, T., Hedlund, K.-O., Strömberg, I., Björklund, H., Hökfelt, T., Melander, T., Seiger, Å. & Strömberg, I. 1988. The innervation apparatus of the rodent iris. In: Handbook of Chemical Neuroanatomy, Vol. 6 The Peripheral Nervous System , pp. 545-597 (A. Björklund, T. Hökfelt and C. Owman, eds.).

Elsevier.

198 Olson, L., Henschen, A., Eriksdotter-Nilsson, M., Strömberg, I., Wetmore, C., Ernfors, P., Friedman, W., Persson, H. & Ebendal, T. 1990. The expanding role of nerve growth factor in the nervous system. In: Growth Factors: From Genes to Clinical Application (Vicki R. Sara et al. eds.) Raven Press, New York, pp. 167-177.

199 Söderström, S., Hallböök, C.F., Ibáñez, C.F., Persson, H & Ebendal, T. 1990. Recombinant human ß-nerve growth factor (NGF): Biological activity and properties in an enzyme immunoassay. J. Neurosci. Res. 27: 665-677.

201 Olson, L., Backlund, E.-O., Ebendal, T., Freedman, R., Hamberger, B., Hansson, P., Hoffer, B., Lindblom, U., Meyerson, B., Strömberg, I., Sydow, O. & Seiger, Å. 1991. Intraputaminal infusion of nerve growth factor to support adrenal medullary autografts in Parkinson's disease: One-year follow-up of first clinical trial. Arch. Neurol. 48: 373-381.

202 Ibáñez, C. F., Hallböök, F., Söderström, S., Ebendal, T. & Persson, H. 1991. Biological and immunological properties of recombinant human, rat and chicken nerve growth factors: a comparative study. J. Neurochem. 57: 1033-1041.

203 Lärkfors, L., Oskarsson, A., Sundberg, J. & Ebendal, T. 1991. Methylmercury induced alterations in the nerve growth factor level in the developing brain. Dev. Brain Res. 62: 287-291.

208 Ebendal, T., Söderström, S., Hallböök, F., Ernfors, P., Ibáñes, C.F., Persson, H., Wetmore, C., Strömberg, I. & Olson, L. 1991. Human nerve growth factor: Biological and immunological activities, and clinical possibilities in neurodegenerative disease. In: Plasticity and Regeneration of the Nervous System (P. S. Timiras, A. Privat, E. Giacobini, J. Lauder and A. Vernadakis, eds.), Adv. Exp. Med. Biol. 296, pp. 207-225. Plenum Publ., N.Y.

212 Ibáñez, C.F., Ebendal, T. & Persson, H. 1991. Chimeric molecules with multiple neurotrophic activities reveal structural elements determining the specificities of NGF and BDNF. EMBO J. 10: 2105-2110.

213 Olson, L., Nordberg, A., von Holst, H., Bäckman, L., Ebendal, T., Alafuzoff, I., Amberla, K., Hartvig, P., Herlitz, A., Lilja, A., Lundqvist, H., Långström, B., Meyerson, B., Persson, A., Viitanen, M., Winblad, B. & Seiger, Å. 1992. Nerve growth factor affects ^{11}C-nicotine binding, blood flow, EEG, and verbal episodic memory in an Alzheimer patient (Case Report). J. Neural Transm. (P-D Sect.) 4: 79-95.

214 Hallböök, F., Ibáñez, C. F., Ebendal, T. & Persson, H. 1993. Cellular localization of brain-derived neurotrophic factor and neurotrophin-3 mRNA expression in the early chicken embryo. Eur. J. Neurosci. 5: 1-14.

215 Olson, L., Wetmore, C., Strömberg, I. & Ebendal, T. 1991. Endogenous and exogenous nerve growth factor in the central nervous system. In: Volume Transmission in the Brain: Novel Mechanisms for Neural Transmission (K. Fuxe and L. F. Agnati, eds.), pp. 455-462. Raven Press, Ltd., New York.

216 Dimberg, Y., Tottmar, O., Aspberg, A., Ebendal, T., Johansson, K.-J. &

Walinder, G. 1992. Effects of low-dose X-irradiation on mouse-brain aggregation cultures. Int. J. Radiat. Biol. 61: 355-363.

217 Ebendal, T., Persson, H. & Olson, L. 1991. New directions in NGF experimental therapy of Alzheimer's disease. In: Cholinergic Basis for Alzheimer Therapy (R. Becker and E. Giacobini, eds.), pp. 468-473. Birkhäuser, Boston.

222 Parvinen, M., Pelto-Hoikko, M., Söder, O., Schultz, R., Kaipia, A., Mali, P., Toppari, J., Lönnerberg, P., Ritzen, E. M., Ebendal, T., Olson, L., Hökfelt, T. & Persson, H. 1992. Expression of β-nerve growth factor and its receptor in rat seminiferous epithelium - specific function at the onset of meiosis. J. Cell Biol. 117: 629-641.

223 Vazquez, E. M. & Ebendal, T. 1991. Messenger RNAs for *trk* and the low-affinity NGF receptor in rat basal forebrain. NeuroReport 2: 593-596.

224 Ibáñez, C. F., Ebendal, T., Barbany, G., Murray-Rust, J., Blundell, T. L. & Persson, H. 1992. Disruption of the low-affinity receptor-binding site in nerve growth factor allows neuronal survival and differentiation by binding to the *trk* gene product. Cell 69: 329-341.

225 Carri, N. G., Rubin, K., Gullberg, D. & Ebendal, T. 1992. Neuritogenesis on collagen substrates. Involvement of integrin-like matrix receptors in retinal fibre outgrowth on collagen. Int. J. Dev. Neurosci. 10: 393-405.

227 Ekblom, J., Ebendal, T., Gillberg, P-G., Nordhage, I. & Aquilonius, S-M. 1992. Quantitative autoradiography of high-affinity nerve growth factor receptors in various segments of the human spinal cord fails to demonstrate alterations in amyotrophic lateral sclerosis. Neurodegeneration 1: 163-168.

228 Henriksson, B. G., Söderström, S., Gower, A. J., Ebendal, T., Winblad, B. & Mohammed, A. H. 1992. Hippocampal nerve growth factor levels are related to spatial learning ability in aged rats. Behav. Brain Res. 48: 15-20.

229 Persson, H., Ernfors, P., Ibáñez, C. F., Hallböök, F., Friedman, W. J., Merlio, J-P., Lindvall, O., Bengzon, J., Lindefors, N., Ebendal, T. & Olson, L. 1992. Neurotrophins and their receptors. In: "Gene Transfer and Therapy in the Nervous System". (Fondation Ipsen Meeting). (Eds. F. Gage and W. Cristen), pp 180-200. Springer Verlag, Berlin-Heidelberg.

230 Ebendal, T. 1992. Function and evolution of the NGF family and its receptors. J. Neurosci. Res. 32: 461-470.

231 Lorigados, L., Söderström, S. & Ebendal, T. 1992. Two-site enzyme immunoassay for bNGF applied to human patient sera. J. Neurosci. Res. 32: 329-339.

232 Carri, N. G., Richardson, P. M. & Ebendal, T. 1994. Choroid coat extract and ciliary neurotrophic factor strongly promote neurite outgrowth in the embryonic chick retina. Int. J. Dev. Neurosci. 12: 567-578.

235 Söderström, S. & Ebendal, T. 1995. *In vitro* toxicity of methyl mercury: effects on nerve growth factor (NGF)-responsive neurons and on NGF synthesis in fibroblasts. Toxicol. Lett. 75: 133-144.

236 Palmer, M. R., Eriksdotter-Nilsson, M., Henschen, A., Ebendal, T. & Olson, L.

1993. Nerve growth factor-induced excitation of selected neurons in the brain which is blocked by a low-affinity receptor antibody. Exp. Brain Res. 93: 226-230.

237 Söderström, S., Fredriksson, A., Dencker, L. & Ebendal, T. 1995. The effect of mercury vapour on cholinergic neurons in the fetal brain: studies on the expression of nerve growth factor and its high- and low-affinity receptors. Dev. Brain Res. 85: 96-108.

239 Bengzon, J., Söderström, S., Kokaia, Z., Kokaia, M., Ernfors, P., Persson, H., Ebendal, T. & Lindvall, O. 1992. Widespread increase of nerve growth factor protein in the rat forebrain after kindling-induced seizures. Brain Res. 587: 338-342.

241 Williams, R., Bäckström, A., Ebendal, T. & Hallböök, F. 1993. Molecular cloning and cellular localization of *trk*C in the chicken embryo. Dev. Brain Res. 75: 235-252.

242 Williams, R., Bäckström, A., Kullander, K., Hallböök, F. & Ebendal, T. 1995. Developmentally-regulated expression of mRNA for neurotrophin high-affinity (*trk*) receptors within chick trigeminal sensory neurons. Eur. J. Neurosci. 7: 116-128.

243 Kullander, K. & Ebendal, T. 1994. Neurotrophin-3 acquires NGF-like activity after exchange to five NGF amino acid residues: Molecular analysis of the sites in NGF mediating the specific interaction with the NGF high affinity receptor. J. Neurosci. Res. 39: 195-210.

244 Durbeej, M., Söderström, S., Ebendal, T. & Ekblom, P. 1993. Differential expression of neurotrophin receptors during renal development. Development 119: 977-989.

249 Granholm, A.-C., Bäckman, C., Bloom, F., Ebendal, T., Gerhardt, G., Hoffer, B., Mackerlova, L., Olson, L., Söderström, S., Walus, L. R. & Friden, P. M. 1994. NGF and anti-transferrin receptor antibody conjugate - Short and long-term effects on survival of cholinergic neurons in intraocular septal transplants. J. Pharmacol. Exp. Ther. 268: 448-459.

250 Jazin, E. E., Zhang, X., Söderström, S., Williams, R., Hökfelt, T., Ebendal, T. & Larhammar, D. 1993. Expression of peptide YY and mRNA for the NPY/PYY receptor of the Y1 subtype in dorsal root ganglia during rat embryogenesis. Dev. Brain Res. 76:105-113.

251 Schwarting, R.K.W., Pei, G., Söderström, S., Ebendal, T. & Huston, J.P. 1994. Unilateral stimulation or removal of rat vibrissae: analysis of nerve growth factor and tyrosine hydroxylase mRNA in the brain. Behav. Brain Res. 60: 63-71.

252 Humpel, C., Ebendal, T., Cao, Y., & Olson, L. 1993. Pentylenetetrazol seizures increase pro-nerve growth factor-like immunoreactivity in the reticular thalamic nucleus and nerve growth factor mRNA in the dentate gyrus. J. Neurosci. Res. 35: 419-427.

253 Ekblom, J., Jossan, S. S., Ebendal, T., Söderström, T., Oreland, L. & Aquilonius, S.-M. 1994. mRNA expression of neurotrophins and members of the *trk* family in the rat brain after treatment with L-deprenyl. Acta Neurol. Scand. 89: 147-148.

255 Granholm, A-C., Biddle, P. T., Bäckman, C., Ebendal, T., Gerhardt, G., Hoffer, B., Mackerlova, L., Olson, L., Söderström, S., Walus, L. & Friden, P. 1994. Peripheral administration of nerve growth factor conjugated to an anti-transferrin receptor antibody increases cholinergic neuron survival in intraocular forebrain transplants. In "Methods in Neurosciences". Providing pharmacological access to the brain: Alternate

approaches. Vol. 21 (T.R. Flanagan, D.F. Emerich and S. Vinn, Eds.), pp. 71-92. Academic Press, N.Y.

256 Seiger, Å., Nordberg, A., von Holst, H., Bäckman, L., Ebendal, T., Alafuzoff, I., Amberla, K., Hartvig, P., Herlitz, A., Lilja, A., Lundqvist, H., Långström, B., Meyerson, B., Persson, A., Viitanen, M., Winblad, B. & Olson, L. 1993. Intracranial infusion of purified nerve growth factor to an Alzheimer patient: The first attempt of possible future treatment strategy. In: Alzheimer's Disease: Animal Models and Clinical Perspectives. (A. Mohammed, B. Henriksson, B. Winblad and P. Södersten, Eds.), Behav. Brain Res. 57: 255-261.

265 Bartheld, C. S. von, Schober, A., Kinoshita, Y., Williams, R., Ebendal, T. & Bothwell, M. 1995. Noradrenergic neurons in the locus coeruleus of birds express TrkA, transport NGF and respond to NGF. J. Neurosci. 15: 2225-2239.

266 Lorigados, L., Molina, H., Serrano, T., Pavón, N., Robinson, M.A., Alvarez, L., Söderström, S. & Ebendal, T. 1995. Evolutive levels of NGF in neurodegenerative disorders. Mol. Chem. Neuropathol. 24: 231-234.

269 Ebendal, T., Tomac, A., Hoffer, B. J. & Olson, L. 1995. Glial cell line-derived neurotrophic factor (GDNF) stimulates fibre formation and survival in cultured neurons from peripheral autonomic ganglia. J. Neurosci. Res. 40: 276-284.

276 Hallböök, F., Bäckström, A., Kullander, K., Ebendal, T. & Carri, N. 1996. Expression of neurotrophins and trk receptors in the avian retina. J. Comp. Neurol. 364: 664-676.

277 Humpel, C., Lindqvist, E., Söderström, S., Kylberg, A., Ebendal, T. & Olson, L. 1995. Monitoring release of neurotrophic activity in the brains of awake rats. Science 269: 552-554.

278 Bäckman, C., Biddle, P. T., Ebendal, T., Friden, P., Gerhardt, G., Henry, M., Mackerlova, L., Söderström, S., Strömberg, I., Walus, L., Hoffer, B. J. & Granholm, A.-Ch. 1995. Effects of transferrin receptor antibody-NGF conjugate on young and aged septal transplants *in oculo*. Exp. Neurol. 132: 1-15.

279 Pei, G. & Ebendal, T. 1995. Specific lesions in the extrapyramidal system of the rat brain induced by 3-nitropropionic acid (3-NPA). Exp. Neurol. 132: 105-115.

280 Hallböök, F., Bäckström, A., Kullander, K., Kylberg, A., Williams, R. & Ebendal, T. 1995. Neurotrophins and their receptors in chicken neuronal development. Int. J. Dev. Biol. 39: 855-868.

281 Holst, A. von, Rodriguez-Tébar, A., Michaille, J.J., Dhouailly, D., Bäckström, A., Ebendal, T. & Rohrer, H. 1995. Retinoic acid-mediated increase in *Trk*A expression is sufficient to elicit NGF-dependent survival of sympathetic neurons. Mol. Cell. Neurosci. 6: 185-198.

282 Sydow, O., Hansson, P., Young, D., Meyerson, B., Backlund, E-O., Ebendal, T., Farnebo, L. O., Freedman, R., Hamberger, B., Hoffer, B., Seiger, Å., Strömberg, I., & Olson, L. 1995. Long-term beneficial effects of adrenal medullary autografts supported by nerve growth factor in Parkinson's

disease. Eur. J. Neurol. 2: 445-454.

283 Tomac, A., Widenfalk, J., Lin, L-F. H., Kohno, T., Ebendal, T., Hoffer, B. J. & Olson, L. 1995. Retrograde axonal transport of glial cell line-derived neurotrophic factor in the adult nigrostriatal system suggests a trophic role in the adult. Proc. Natl. Acad. Sci. USA 92: 8274-8278.

285 Söderström, S. & Ebendal, S. 1995. Levels of neurotrophin-3 protein in the rat brain as determined by enzyme immunoassay show a pattern distinct from nerve growth factor. Neurosci. Lett. 189: 5-8.

286 Hallböök, F., Ebendal, T. & Carri, N.G. 1995. Neurotrophins in the developing avian visual system. In: "Life and Death in the Nervous System. Role of Neurotrophic Factors and their Receptors". Wenner-Gren Foundation International Series Vol. 67 (C. Ibáñez, T. Hökfelt, L. Olson, K. Fuxe, H. Jörnvall and D. Ottoson, Eds.), pp. 143 - 153. Stockholm, Sweden.

287 Williams, R., Bäckström, A. & Ebendal, T. 1995. Embryonic expression of neurotrophin high-affinity (*trk*) receptor mRNAs within sensory neurons: chicken development as a model. In: "Life and Death in the Nervous System. Role of Neurotrophic Factors and their Receptors". Wenner-Gren Foundation International Series Vol. 67 (C. Ibáñez, T. Hökfelt, L. Olson, K. Fuxe, H. Jörnvall and D. Ottoson, Eds), pp. 113 - 130. Stockholm, Sweden.

290 Martínez-Serrano, A., Fischer, W., Söderström, S., Ebendal, T. & Björklund, A. 1996. Long-term functional recovery from age-induced spatial memory impairments by nerve growth factor gene transfer to the rat basal forebrain. Proc. Natl. Acad. Sci. USA 93: 6355-6360.

291 Durbeej, M., Fecker, L., Hjalt, T., Zhang, H.-Y., Salmivirta, K., Klein, G., Timpl, R., Sorokin, L., Ebendal, T., Ekblom, P. & Ekblom, M. 1996. Expression of laminin $\alpha 1$, $\alpha 5$ and $\beta 2$ chains during embryogenesis of the kidney and vasculature. Matrix Biol. 15: 397-413.

292 Hasenöhrl, R.U., Söderström, S., Mohammed, A.H., Ebendal, T. & Huston, J.P. 1997. Reciprocal changes in expression of mRNA for nerve growth factor and its receptors TrkA and LNGFR in brain of aged rats in relation to maze learning deficits. Exp. Brain Res. 114: 205-213.

293 Bengtsson, H., Söderström, S. & Ebendal, T. 1995. Expression of activin receptors type I and II only partially overlaps in the nervous system. NeuroReport 7: 113-116.

294 Estenne-Bouhtou, G., Kullander, K., Karlsson, M., Ebendal, T., Hacksell, U., & Luthman, K. 1996. Design, synthesis, tandem mass spectrometric sequencing, and biological activity of NGF-mimetics. Int. J. Peptide Prot. Res. 48: 337-346.

296 Williams, R. & Ebendal, T. 1995. Neurotrophin receptor expression during development of the chick spinal sensory ganglion. NeuroReport 6: 2277-2282.

300 McGrath, J.P., Cao, X., Schutz, A., Lynch, A., Ebendal, T., Coloma, M.J.., Morrison, S.L. & Putney, S.D. 1997. Bifunctional fusion between nerve growth factor and a transferrin receptor antibody. J. Neurosci. Res. 47: 123-133.

301 Nosrat, C.A., Ebendal, T. & Olson, L., 1996. Differential expression of brain-derived neurotrophic factor and neurotrophin 3 mRNA in lingual papillae and taste buds

indicates roles in gustatory and somatosensory innervation. J. Comp. Neurol. 376: 587-602.

j303 Aoyagi, K., Bergsten, P., Eriksson, U.J., Ebendal, T. & Hellerström, C. 1997. *In vitro* regulation of insulin release and biosynthesis of fetal rat pancreatic cells explanted on pregnancy day 16. Biol. Neonate 71: 60-68.

305 Kobayashi, S., Ögren, S.-O., Ebendal, T. & Olson, L. 1997. Intraventricular infusion of NGF, but not BDNF, induces rapid motor activation that is inhibited by nicotinic receptor antagonists. Exp. Brain Res. 116: 315-325.

306 Lewén, A., Söderström, S., Hillered, L. & Ebendal, T. 1997. Expression of serine/threonine kinase receptors in traumatic brain. NeuroReport 8: 475-479.

307 Söderström. S., Bengtsson, H. & Ebendal, T. 1996. Expression of serine-threonine protein kinase receptors including the bone morphogenetic factor type II receptor in developing and adult rat brain. Cell Tiss. Res. 286: 269-279.

309 Bäckström, A., Söderström, S., Kylberg, A. & Ebendal, T. 1996. Molecular cloning of the chicken trkA and its expression in early peripheral ganglia. J. Neurosci. Res. 45: 67-81.

311 Nosrat, C., Tomac, A., Lindqvist, E., Lindskog, S., Humpel, C., Strömberg, I., Ebendal, T., Hoffer, B.J. & Olson, L. 1996. Cellular expression of GDNF mRNA suggests multiple functions inside and outside the nervous system. Cell Tissue Res. 286: 191-207.

312 Lorentzon, M., Hoffer, B., Ebendal, T., Olson, L. & Tomac, A. 1996. Habrec1, a novel serine/threonine kinase TGFβ type I-like receptor, has a specific cellular expression suggesting function in the developing organism and adult brain. Exp. Neurol. 140: 351-360.

313 Humpel, C., Ebendal., T. & Olson, L. 1996. Microdialysis: a way to study in vivo of neurotrophic bioactivity: A critical summary. J. Mol. Med. 74: 523-526.

318 Kullander, K., Kaplan, D. & Ebendal, T. 1997. Two restricted sites on the NGF molecule independently determine specific TrkA receptor binding and activation. J. Biol. Chem. 272: 9300-9307.

319 Carri, N.G., Bengtsson, H., Charette, M.F. & Ebendal, T. 1998. BMP receptor II expression and OP-1 effects in developing chicken retinal explants. NeuroReport 9 (in press).

320 Bäckström, A., Söderström, S. & Ebendal, T. 1997. Cloning of a new chicken trkA extracellular isoform and its mRNA expression in E9 sensory and autonomic ganglia. Int. J. Dev. Neurosci. 15: 275-284.

321 Dimberg, Y., Vazquez, E. M., Söderström, S. & Ebendal, T. 1997. Effects of irradiation on nerve growth factor in the developing mouse brain. Toxicol. Lett. 90: 35-43.

322 Nordberg, A., Almkvist, O., Amberla, K., Basun, H., Corder, B., Ebendal, T., Hellström, C.-G., Hartwig, P., Hellström-Lindahl, E., Jelic, V., Jönhagen, M., Lannfelt, L., Lehman, W., Långström, B., Lundquist, H., Meurling, L., Meyersson, B., Olson, L., Seiger, Å., Valind, S., Viitanen, M., Wahlund, L.-O. & Winblad, B. 1997. Responders and non-responders to tacrine, ondansetron and NGF treatment in Alzheimer patients evaluated by positron emission tomography and APOE genotype. In: Alzheimer's

Disease: Biology, Diagnosis and Therapeutics (eds. Iqbal et al.), John Wiley & Sons, pp. 647-653.

323 Kobayashi, S., Ögren, S.-O., Ebendal, T. & Olson, L. 1997. Dopamine receptor antagonists block NGF-induced hyperactivity. Eur. J. Pharmacol. 326: 1-5.

324 Lindeberg, J., Klint, P., Bäckström, A., Williams, R. & Ebendal, T. 1997. Identification of a chicken homologue in the Brn-3 subfamily of POU-transcription factors. Dev. Brain Res. 100: 169-182.

325 Granholm, A.-C., Albeck, D., Bäckman, C., Curtis, M., Ebendal, T., Friden, P., Henry, M., Hoffer, B., Kordower, J., Rose, G.M., Söderström, S. and Bartus, R.T. 1998. A non-invasive system for delivering neural growth factors across the blood-brain barrier: a review. Rev. Neurosci. (in press).

326 Kullander, K., Kylberg, A. & Ebendal, T. 1997. Specificity of neurotrophin-3 determined by loss-of-function mutagenesis. J. Neurosci. Res. 50: 496-503.

327 Jazin, E.E., Söderström, S., Ebendal., T. & Larhammar, D. 1997. Embryonic expression of the mRNA for the rat homologue of the fusin/CXCR-4 HIV-1 co-receptor. J. Neuroimmunol. 79: 148-154.

329 Riikonen, R., Söderström, S., Vanhala, R., Ebendal, T. & Lindholm, D.B. 1997. West's syndrome: Cerebrospinal fluid nerve growth factor and effect of ACTH. Pediatr. Neurol. 17: 224-229.

330 Kobayashi, S., Ögren, S.-O., Ebendal, T. & Olson, L. 1997. Intraventricular injection of NGF, but not BDNF, induces rapid motor activation that is inhibited by nicotinic receptor antagonists. Exp. Brain Res. 116: 315-325.

331 Nosrat, C., Fried, K., Ebendal, T., & Olson, L. 1998. NGF, BDNF, NT3, NT4 and GDNF in tooth development. Eur. J. Oral. Sci. 106 (suppl. 1): 94-99.

332 Kadari, A., Windisch, J.M., Ebendal, T., Schneider, R. & Humpel, C. 1997. Cell death of adult pyramidal CA1 neurons after intraventricular injection of a novel peptide derived from *trk*A. J. Neurosci. Res. 50: 402-412.

337 Eriksdotter Jönhagen, M., Amberlaa, K., Bäckman, L., Ebendal, T., Meyerson, B., Nordberg, A., Olson, L., Seiger, Å., Shigeta, M., Theodorson, E., Wahlund, L.-O., Viitanen, M. & Winblad, B. 1998. Intracerebroventricular infusion of nerve growth factor in three patients with Alzheimer's disease. Dementia Geriatric Cogn. Disord. (in press).

338 Ebendal, T., Bengtsson, H. & Söderström, S. 1998. Bone morphogenetic proteins and their receptors: potential functions in the brain. J. Neurosci. Res. 51: 139-146.

Journal of Neuroscience Research 40:276–284 (1995)

Rapid Communication

Glial Cell Line-Derived Neurotrophic Factor Stimulates Fiber Formation and Survival in Cultured Neurons From Peripheral Autonomic Ganglia

T. Ebendal, A. Tomac, B.J. Hoffer, and L. Olson
Department of Developmental Neuroscience, Biomedical Center, Uppsala University, Uppsala (T.E.), and Department of Neuroscience, Karolinska Institute, Stockholm (A.T., B.J.H., L.O.), Sweden; Department of Pharmacology, University of Colorado, Denver (B.J.H.)

Human recombinant glial cell line-derived neurotrophic factor (GDNF) was tested for its ability to stimulate fiber formation and neuron survival in primary cultures of peripheral ganglia dissected from the chicken embryo. GDNF, first characterized by its actions on central nervous system (CNS) neurons, had a marked stimulatory effect on fiber outgrowth in sympathetic and ciliary ganglia. Weaker responses were evoked in sensory spinal and nodose ganglia and in the ganglion of Remak. In addition, survival of neurons from the sympathetic and ciliary ganglia was stimulated by GDNF at 50 ng/ml. The effects were not mimicked by the distant but related protein transforming growth factor beta 1 (TGFβ1). The profile of neurons stimulated by GDNF is also distinct from the patterns of stimulation shown by nerve growth factor (NGF), stimulating strongly sympathetic but not ciliary ganglia, and ciliary neurotrophic factor (CNTF), stimulating mainly the ciliary ganglion. Moreover, using in situ hybridization histochemistry, GDNF was demonstrated to be present in the pineal gland in the newborn rat, a target organ for sympathetic innervation. The present results suggest that GDNF is likely to act upon receptors present in several autonomic and sensory neuronal populations. GDNF may serve to support fiber outgrowth and cell survival in peripheral ganglia, adding yet one more trophic factor to the list of specific proteins controlling development and maintenance of the peripheral nervous system.

Key words: trophic factor, TGFβ, chicken embryo, tissue culture, bioassay

INTRODUCTION

Development, selective survival, and function of neurons are regulated by cellular interactions mediated by a host of neurotrophic molecules. Thus, proteins with a well-characterized ability to support neurons include the family of neurotrophins [nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins-3 and -4 (NT-3 and NT-4)], ciliary neurotrophic factor (CNTF), acidic and basic fibroblast growth factors (aFGF and bFGF, respectively), and insulin-like growth factors (IGF-1 and IGF-2).

A recent addition to the list of proteins with trophic effects on neurons is glial cell line-derived neurotrophic factor (GDNF) (Lin et al., 1993). GDNF was initially documented to support survival, differentiation, and high-affinity dopamine uptake in fetal dopamine neurons from the ventral mesencephalon in vitro. The active molecule was purified and partially sequenced and subsequently DNA clones encoding the novel neurotrophic factor were isolated from rat cDNA and human genomic DNA (Lin et al., 1993). Analysis of the sequences obtained showed that GDNF, a 134-amino-acid protein in its mature form, is a member of the transforming growth factor beta (TGFβ) superfamily of growth and transforming factors (see review by Massagué, 1990) but is not closely related to any of the many previously known

Received September 19, 1994; revised November 7, 1994; accepted November 8, 1994.

Address reprint requests to Dr. Ted Ebendal, Department of Developmental Neuroscience, Biomedical Center, Uppsala University, Box 587, S-751 23 Uppsala, Sweden.

members of this family (Burt, 1992; Lin et al., 1993; Burt and Law, 1994). When expressed as a recombinant protein produced in bacteria, the GDNF, after proper refolding (Lin et al., 1993) had the expected dopaminotrophic activity originally described in the glial cell line from which it was derived (Schubert et al., 1974). The TGFβ proteins, the neurotrophins, and the platelet-derived growth factors A and B (PDGFs) all share some protomeric structural motifs rendering them members of the cystine-knot superfamily of growth factors (McDonald and Hendrickson, 1993). GDNF, like the other cystine-knot proteins, form homo- or heterodimers that are the biologically active entities. It is thus interesting that neurotrophic activities have been ascribed not only to NGF and the other neurotrophins, but also to PDGF and to TGFβs. Very recently, the structural characterization of human chorionic gonadotropin (hCG) has shown that it is also a member of the cysteine-knot family of proteins (Lapthorn et al., 1994) but the neurotrophic activity of hCG has not yet been reported.

So far, studies of the effects of GDNF have involved neurons of the central nervous system (CNS) such as the mesencephalic dopamine neurons and α-motoneurons (Lin et al., 1993). In addition, GDNF is expressed in the rat striatum and in other areas of the brain during development (Schaar et al., 1993; Strömberg et al., 1993). Moreover, in the adult rat brain, kainate-induced epileptic seizures induce GDNF expression in the granule cells of the adult dentate gyrus (Humpel et al., 1994), and pilocarpine-induced seizures lead to upregulation of GDNF in the striatum (Schmidt-Kastner et al., 1994).

In the present report we demonstrate that GDNF also has the ability to stimulate peripheral neurons. We document neurotrophic effects of GDNF in a series of sympathetic and parasympathetic autonomic as well as some sensory peripheral neurons in culture. It is shown that GDNF has substantial trophic effects, particularly on some autonomic neuron populations, and that these patterns of stimulation are distinct from those evoked by the neurotrophins (NGF, BDNF, NT-3, and NT-4) as well as CNTF and TGFβ1.

MATERIALS AND METHODS

Ganglia from chicken embryos at day 9 of incubation were explanted as intact ganglia for a fiber-outgrowth assay (Ebendal et al., 1978; Hedlund and Ebendal, 1978; Ebendal et al., 1980, 1984; Ebendal, 1989) or, in the case of the sympathetic paravertebral trunk ganglia and the ciliary ganglion, dissociated for a neuron-survival assay (Ebendal et al., 1985; Kullander and Ebendal, 1994). Sympathetic paravertebral ganglia and sensory spinal ganglia (dorsal root ganglia) were dissected from the lumbosacral region of the embryo. The ciliary ganglion was taken from the orbit, the nodose ganglion from the vagus nerve rostral to the heart. Finally, the ganglion of Remak was isolated from the mesorectum of the embryo (Hedlund and Ebendal, 1978). The effects of GDNF in cultures of the sensory trigeminal and spinal (dorsal root) ganglia were also tested. Ganglia or dissociated neurons were placed in collagen gels for culture as detailed by Ebendal (1989). All findings were repeated at least twice in independent experiments yielding the same results. The basic finding of a strong GDNF stimulation of the sympathetic ganlion has been repeated in more than 25 cultures established over a period of more than 6 months in our laboratory. Likewise, the survival data are based on counting of several hundred neurons on several occasions.

Recombinant human GDNF was obtained from Synergen, Inc. (Boulder, CO). The protein was produced in bacteria, refolded to yield an active molecule, and purified as a non-glycosylated disulfide-bonded homodimer as described (Lin et al., 1993). Human recombinant TGFβ1 was obtained from Boehringer Mannheim (Darmstadt, Germany). Both proteins were aliquoted to avoid repeated freeze-thawing cycles and added to the culture medium at the final concentrations indicated for each experiment. For positive effect on neuron survival, purified mouse βNGF (Ebendal et al., 1984) was added to sympathetic neurons at 5 ng/ml. To support ciliary neuron survival, an extract of the choroid coat including the pigment epithelium from the 18-day-old chicken embryo was added at a final concentration of 200 μg of total protein/ml of medium (Ebendal, 1987).

Ganglia and dissociated neurons were observed under darkfield or phase contrast optics, fiber outgrowths observed, neurons counted, and microphotographs taken after 2 days in culture.

For in situ hybridization, E17 rat fetuses were taken from pregnant Sprague-Dawley rats that had been killed by neck dislocation under deep ether anesthesia. The heads of the fetuses were rapidely frozen on dry ice. Newborn rats were killed by decapitation and the heads frozen on blocks of dry ice. Cryostat sections (14 μm thick) of the fetal and neonatal brains were cut and placed on coated slides (ProbeOn, Fisher Biotech, Orangeburg, NY). To perform in situ hybridization (Dagerlind et al., 1992), sections were thawed and hybridized with two antisense oligonucleotide probes (both 50-meres, positions corresponding to nucleotides 456–505 and 540–589, respectively, in the sequence deposited under GenBank accession number L15305; see Lin et al., 1993). These two oligonucleotide probes had no similarities to sequences deposited in GenBank and generated identical in situ hybridization patterns in tissue. As a negative control, a random probe of the same length and GC content was applied to adjacent sections. This

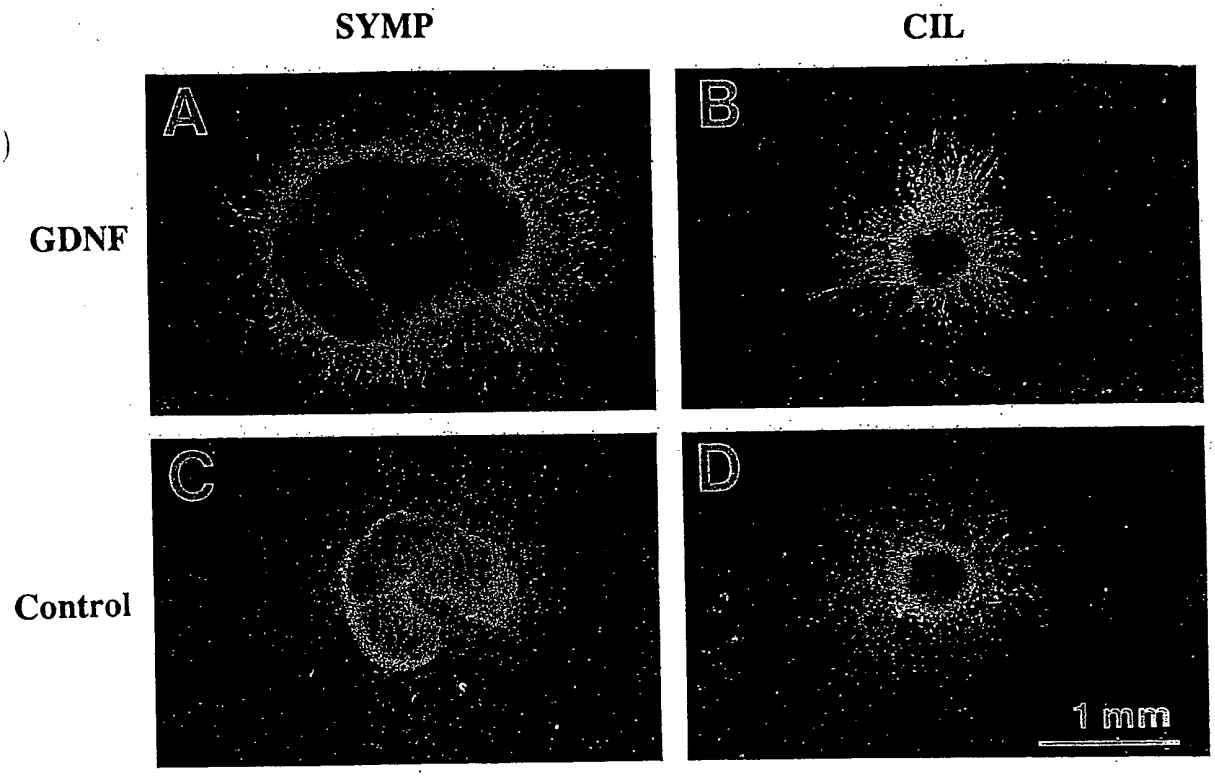


Fig. 1. The response to GDNF in explanted sympathetic and ciliary ganglia. **A:** A sympathetic ganglion cultured for 2 days in the presence of GDNF at 100 ng/ml. A dense, short fiber outgrowth is evident around the ganglion. **B:** The same conditions as in A but with a ciliary ganglion. The formation of fiber fascicles is evident. **C:** A negative control with a sympathetic ganglion cultured for 2 days without the support of GDNF. **D:** A ciliary ganglion in control medium. Darkfield micrographs of living cultures.

random probe did not give rise to any signals above background in examined tissues. The oligoprobes were end labeled with ^{35}S-dATP using terminal deoxyribonucleotidyl transferase. The probes were then purified (Nensorb columns) and applied to the tissue sections at 42°C overnight in a hybridization solution. Following this, the slides were rinsed in 1× SSC at 54°C, dehydrated in a series of ethanols, and air-dried. The slides were dipped in Kodak NTB-2 film emulsion and exposed for 6–8 weeks at −20°C, developed in a photographic developer, fixed, lightly stained with cresyl violet, and mounted. The sections were observed and photographed with dark- and brightfield illumination (Nikon Microphot FX microscope).

RESULTS

GDNF, present at a concentration of 50–100 ng/ml, was consistently found to evoke marked fiber outgrowth in the explanted sympathetic and ciliary ganglia cultured for 2 days (Fig. 1A,B). The outgrowth consisted of dense, short fibers around the sympathetic ganglia. From the ciliary ganglion, GDNF evoked the formation of fairly thick fiber fascicles. The concentrations tested were 0.5, 5, 50, 100, 200, 400, and 1,000 ng/ml of GDNF in the medium and optimum fiber responses were obtained in the sympathetic ganglia with 50 and 100 ng/ml. The resulting outgrowth in the sympathetic ganglion was less prominent than that evoked by NGF at 3–5 ng/ml (Ebendal, 1989), but the fibers were dense and markedly tufted. For comparison, representative NGF-induced outgrowth responses in sympathetic ganglia in this assay can be found in a recent report by Kullander and Ebendal (1994).

The ciliary ganglion responded by fiber outgrowth to GDNF in the range of 5–1,000 ng/ml, thus at a wider range of concentrations than found effective to evoke fiber outgrowth in the sympathetic ganglion. Control cultures of sympathetic and ciliary ganglia without added GDNF were totally devoid of fiber outgrowth (Fig. 1C,D).

Some effects of GDNF on fiber outgrowth were

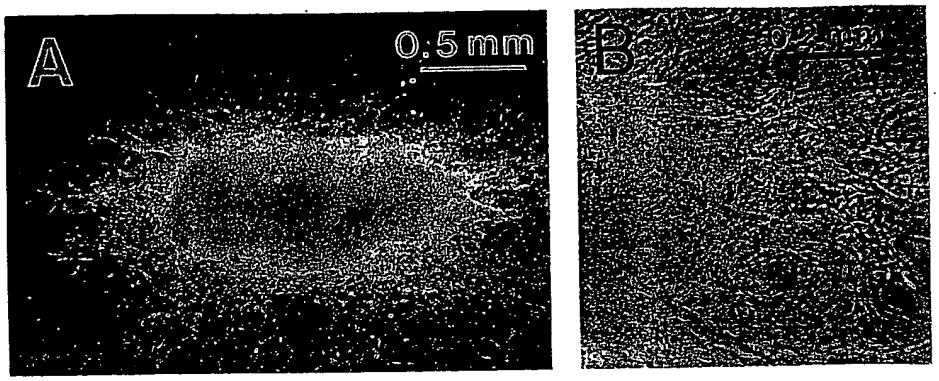


Fig. 2. Effects of GDNF on fiber outgrowth in the nodose ganglion. Some thick fiber fascicles have formed from this sensory ganglion cultured for 2 days with 100 ng/ml of GDNF. **A:** Darkfield overview of the ganglionic explant. **B:** Phase contrast close-up showing the heavily fasciculated fibers induced by GDNF.

also observed in the nodose ganglion (Fig. 2A). A few thick fiber fascicles (Fig. 2B) were formed from this sensory ganglion in response to 100 ng/ml of GDNF over the 2 day culture period. It should be stressed that the outgrowth response was markedly weaker than that evoked by NT-3 at 5–10 ng/ml (for a comparison, see the results obtained in this assay presented by Ernfors et al., 1990) in this epidermal placode-derived ganglion. GDNF also weakly stimulated some fiber outgrowth in the spinal dorsal root ganglia (not shown) and in the trigeminal ganglion (data not shown). The effect of GDNF on fiber outgrowth from Remak's ganglion (data not shown) was only marginal at 50 ng/ml, in contrast to the very strong fiber outgrowth evoked in this ganglion by NT-3 at concentrations of 3–5 ng/ml (Ernfors et al., 1990; Kullander and Ebendal, 1994).

The specificity of the GDNF stimulation in the ganglia was tested by comparisons of neurotrophic responses evoked by TGFβ1. Sympathetic ganglia cultured for 2 days with TGFβ1 at 10 and 100 ng/ml were found to lack fiber outgrowth and thus resemble the control explants in ordinary culture medium (Fig. 3A,B), in contrast to the dense fiber outgrowth formed in response to GDNF at 100 ng/ml (Fig. 3C,D). The specificity of the GDNF stimulation was tested also in the ciliary ganglion. In control medium no formation of fibers occurred (Fig. 4A). The ciliary ganglion likewise showed no response to TGFβ1 at 10 or 100 ng/ml (Fig. 4B) present for 2 days in the culture. In contrast, GDNF present at 100 ng/ml resulted in the formation of dense fiber fascicles (Fig. 4C).

In addition to selective stimulation of fiber outgrowth in some peripheral ganglia, GDNF has survival effects on dissociated ganglionic neurons in culture. A dose-response relationship between survival of sympathetic neurons and the presence of GDNF in the concentration range 1–100 ng/ml is shown in Figure 5A. Half-maximum survival effect was seen at about 50 ng/ml GDNF and represents rescue of nearly half of the seeded neurons. Increasing the dose of GDNF above 100 ng/ml did not further enhance survival rate (data not shown). A similar response was found in the dissociated ciliary neurons stimulated by GDNF (Fig. 5B). For sympathetic neurons, NGF at 5 ng/ml will rescue close to 100% of the neurons. The same is true for ciliary neurons when grown with a choroid extract from the eye of the embryonic day 18 chicken (Ebendal, 1987).

In order to examine the presence of GDNF mRNA in target tissue for peripheral autonomic innervation, we used the rat since the chicken GDNF sequence is not yet known. In situ hybridization using two non-overlapping GDNF specific oligonucleotide probes revealed a strong signal in the pineal gland of the newborn rat (Fig. 6A). The signal appeared to be present over most but not all pinealocytes (Fig. 6B). A similar signal was seen also in the pineal gland of the fetal rat at embryonic day 17 (not shown).

DISCUSSION

In the present report we show that GDNF has a pattern of stimulating ganglionic neuron populations distinct from those described earlier for the neurotrophins NGF, BDNF, NT-3, and NT-4, as well as from that of the ciliary neurotrophic factor CNTF. Thus, the Remak ganglion is strongly stimulated by NT-3 (Ernfors et al., 1990; Kullander and Ebendal, 1994) due to its abundant expression of the tyrosine kinase receptor TrkC (Williams et al., 1993), whereas GDNF had very limited stimulatory action on this ganglion. The sympathetic ganglion was markedly stimulated by GDNF but not to the extent of that seen with NGF. The response involved

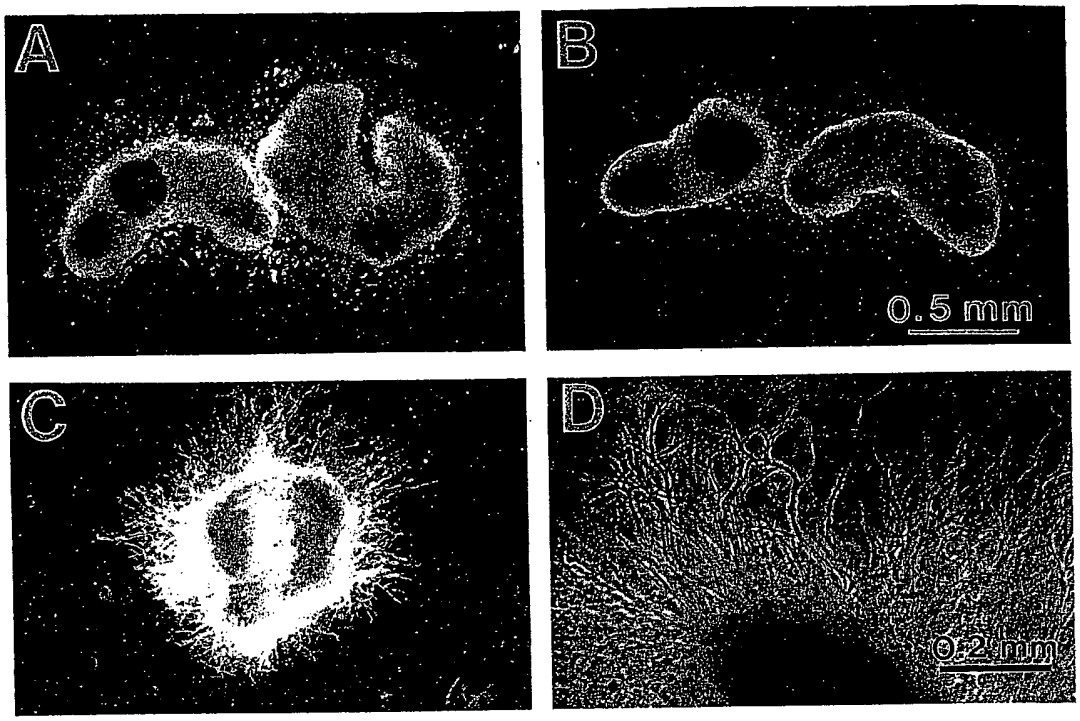


Fig. 3. Specificity of the GDNF stimulation in the sympathetic ganglion. **A:** Sympathetic ganglion in control medium after 2 days of culture. **B:** A sympathetic ganglion cultured for 2 days with TGFβ1 at 100 ng/ml. **C:** Sympathetic ganglion with GDNF at 100 ng/ml present in the medium for the culture period of 2 days. Only with GDNF present are fibers formed. Darkfield microscopy. **D:** Phase contrast detail of fiber outgrowth from a sympathetic ganglion grown for 2 days with GDNF at 100 ng/ml.

both a survival and a fiber outgrowth response. On the other hand, GDNF also stimulated the ciliary ganglion which NGF does not. It remains to be examined whether there are time-dependent switches in the responsiveness to GDNF in the autonomic and sensory neurons examined here in analogy to changing dependency for different neurotrophins found earlier in sensory neurons (Buchmann and Davies, 1993). This could provide insight into potential temporal windows during development when these peripheral neurons depend on GDNF. In situ hybridization has been utilized to study GDNF expression and its relationship to innervation. It has been shown that there is an early expression of GDNF in the brain which is later downregulated (Schaar et al., 1993; Strömberg et al., 1993), and such an expression pattern may argue for developmental regulation of GDNF to serve neurosupportive functions during restricted periods of neurodevelopment.

Embryonic tissue extracts and explants have, in a number of cases, been shown to stimulate ganglionic neurons in culture (Ebendal et al., 1980, 1984, 1985; Ebendal, 1987). Many of these effects have been ascribed to the presence of CNTF, but also to aFGF and bFGF. GDNF is one more factor which may have contributed to these observed stimulatory effects.

In order to examine if GDNF mRNA is present in any target areas for peripheral innervation we used the perinatal rat, since the GDNF gene has not yet been sequenced from the chicken. In view of the marked effects of GDNF on the chicken sympathetic ganglia, we chose to study one target organ for the sympathetic superior cervical ganglion, i.e., the pineal gland. Consistent with the in vitro data, the pineal gland was found to express relatively high levels of GDNF mRNA during late prenatal and early postnatal stages of development (Fig. 6). Thus, GDNF may be one of the target-derived trophic factors stimulating the sympathetic nerves that innervate targets such as the pineal gland.

In the present study, specific activities of GDNF on the responsive peripheral neurons were found in the range of 50–100 ng/ml of the factor. This contrasts with the lower concentrations found to evoke tyrosine hydroxylase (TH) induction in cultured ventral mesencephalic neurons. Thus, Lin et al. (1993) found GDNF to be

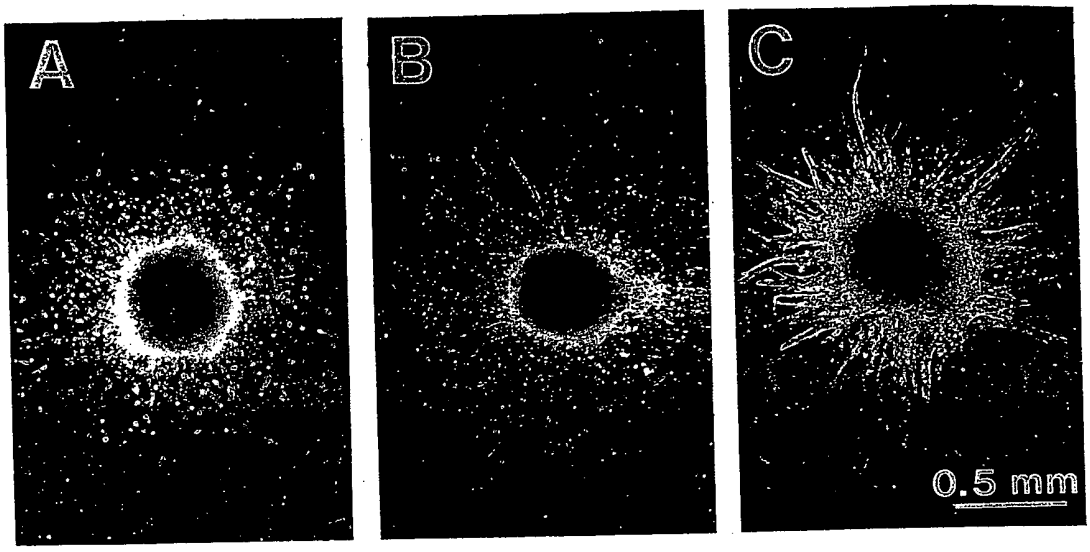


Fig. 4. Specificity of the GDNF stimulation in the ciliary ganglion. **A:** Ganglion grown in control medium for 2 days. No formation of fibers occurs. **B:** A ciliary ganglion showing no response to TGFβ1 at 100 ng/ml. **C:** A ciliary ganglion cultured for 2 days with GDNF present at 100 ng/ml. GDNF, but not TGFβ1, elicited dense fiber fascicles. Darkfield microscopy.

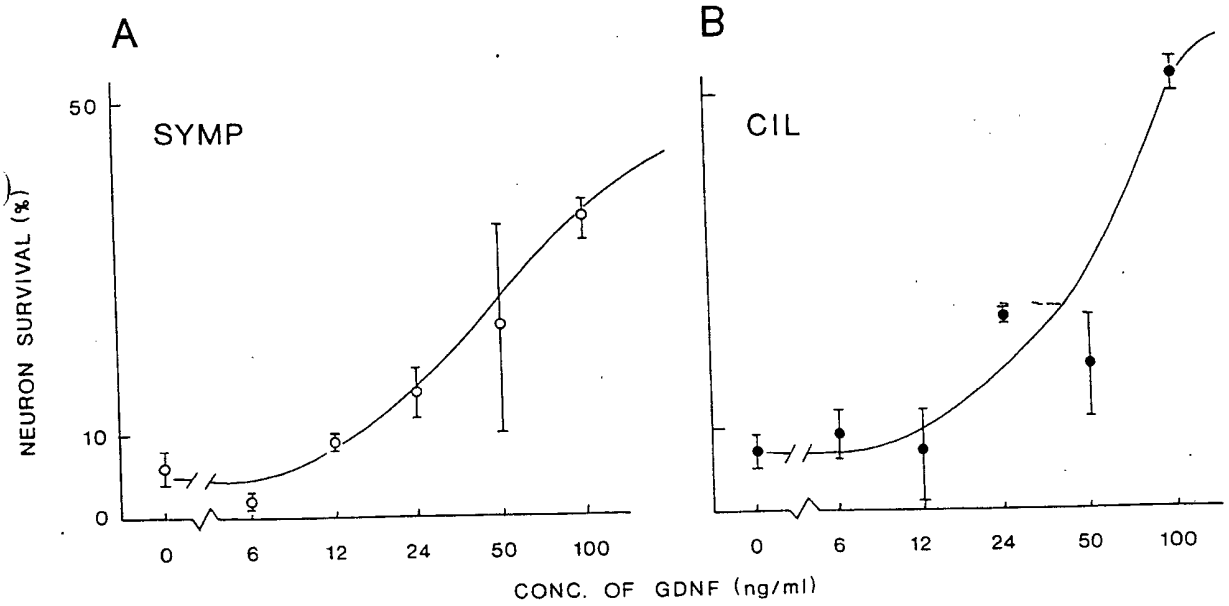


Fig. 5. Survival effect of GDNF on dissociated ganglionic neurons in culture. **A:** Dose-response curve for survival of sympathetic neurons in response to GDNF. **B:** The same experiment shown for ciliary ganglionic neurons. Each graph is based on two independent experiments, with each value based on observations of the survival in several hundred neurons. Mean value and range are shown. Survival is shown relative to the number of initially seeded neurons.

effective at a concentration of 0.04–1 ng/ml, which is in line with what is generally expected for a high-affinity interaction between a growth factor and its specific receptor. It is therefore possible that the present effects are the result of GDNF mimicking a related endogenous growth factor that might act on the peripheral neurons at higher efficiency.

The present experiments showed that GDNF stim-

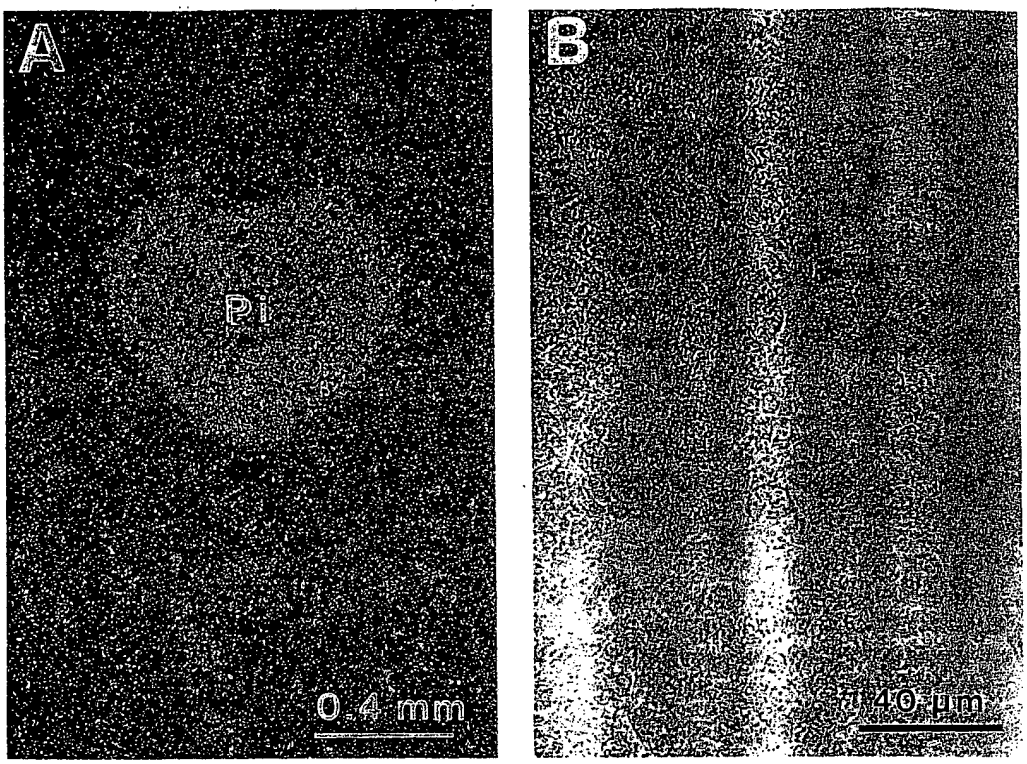


Fig. 6. In situ hybridization with an oligonucleotide probe complementary to rat GDNF mRNA. **A:** Darkfield overview of the pineal gland (Pi) of the newborn rat. **B:** Brightfield micrograph showing hybridization signal over many but not all pinealocytes.

ulated the ciliary ganglion to form nerve fibers in a concentration range of 5–1,000 ng/ml, thus at a wider range of concentrations than was effective for the sympathetic ganglion. This may reflect differences in the growth properties of the ganglia when taken to culture and not necessarily point to different receptor mechanisms. We have earlier found that NT-3 marginally stimulates fiber formation in the ciliary ganglion (Ernfors et al., 1990). The response to GDNF is stronger than that elicited by NT-3 and also appears stronger than the fiber outgrowth response evoked by recombinant CNTF in explanted ciliary ganglia (see Carri et al., 1994).

Before the cloning of GDNF, members of the TGFβ superfamily of growth factors have been considered to possess various neurotrophic and neuron differentiating properties. Thus TGFβ1 promotes the survival of motoneurons in culture, but fails to support the survival of dissociated sympathetic neurons (Martinou et al., 1990), in contrast to the action of GDNF shown here. Activin has been found to stimulate survival in some populations of neurons including the chicken embryo retina (Schubert et al., 1990), but has failed to support the survival of dissociated ciliary ganglionic neurons in an assay similar to the one used here. Again, this contrasts with the supportive effect of GDNF on dissociated ciliary ganglion neurons in the present report.

TGFβ1, -2, and -3 have all been localized in the nervous system using specific peptide antibodies (Flanders et al., 1991). TGFβ1 was mainly found in the meninges, whereas TGFβ2 and TGFβ3 were found in neurons and radial glia. It was also found that TGFβ2 and -3 inhibited the survival of ciliary ganglion neurons in the presence of an eye extract normally supporting these neurons in dissociated cultures (Flanders et al., 1991). This could again indicate the presence of receptors for TGFβs being present on these primary neurons. Supportive of this, TGFβ1 and -2 were found to enhance sensory dorsal root ganglion neuron survival and result in increased levels of substance P (Chalazonitis et al., 1992), actions suggested to be exerted synergistically with NGF. Other additive or synergistic effects of GDNF with the neurotrophins are thus possible, but have yet to be studied.

The present data suggest that a receptor mechanism for GDNF or a related trophic factor is present in developing sympathetic and ciliary neurons and that the receptor specificity is such that GDNF is an agonist ligand whereas TGFβ1 fails to elicit the signaling events lead-

ing to fiber outgrowth or neuron survival in these neurons. Several receptors for the members of the TGFβ family have been identified (see reviews by Massagué, 1992; Lin and Lodish, 1993), but the GDNF receptor, or receptors, remains uncharacterized. It seems likely that the receptor molecules for GDNF are related to the other signaling receptors mediating the actions of the members of the TGFβ superfamily. Activin receptors (Matthews and Vale, 1991; Attisano et al., 1992) and a TGFβ receptor serine/threonine kinase receptor have been cloned (Lin et al., 1992) and classified as type II transmembrane receptors of approximately 75 kDa. Also several type I receptors have been cloned (ten Dijke et al., 1994). It is considered that TGFβ actions are mediated via signal transduction involving the heterodimerization of receptors of the two types and that type II receptors exhibit a higher degree of specificity for their cognate ligands than the type I receptors (ten Dijke et al., 1994). It has recently been shown that the receptors of class II bind the TGFβ and as a second step recruit receptors of type I, which as a result is phosphorylated on serines and threonines as an initial step in a cascade of signaling phosphorylation of downstream proteins (Wrana et al., 1994). Recently, a chicken type II TGFβ receptor has been cloned (Barnett et al., 1994) and has considerable sequence similarity to the mammalian TGFβ receptor II in the kinase domain but is highly divergent in the N-terminal ligand binding part of the receptor. Whether this receptor is mediating the GDNF effects seen presently in peripheral chicken neurons remains to be studied.

Since TGFβ induces the formation of extracellular matrices (Massagué, 1990; Lin and Lodish, 1993) similar effects may be evoked by GDNF. Such a mechanism might be particularly important in the development of the peripheral nervous system where growing axons come into contact with a rich extracellular matrix. It has also been shown that various molecular components of such matrices enhance the formation of fiber outgrowth from the ganglia studied here (Carri et al., 1988). A further possible mechanistic link between GDNF and the extracellular matrix involves the possibility that such matrices bind locally secreted GDNF to enhance nerve growth and neuron differentiation, as has been shown for other members of the TGFβ superfamily (Massagué, 1990; Lin and Lodish, 1993).

The importance of GDNF for sympathetic ganglion development in vivo remains unclear. Null mutations of members of the neurotrophin family in mice suggest that GDNF does not have a function that is redundant to that of the neurotrophins. Thus the superior cervical ganglion will lose neurons in the mice homozygous for the null mutation. However, the temporal relationships have not been defined so the contribution of GDNF to survival of peripheral neurons cannot yet be established. Homozygous NGF null mutant mice (Crowley et al., 1994) show marked reductions in the sympathetic superior cervical ganglion, with up to 80% reduction of the ganglionic volume, losses of neurons, and the presence of abundant pycnotic nuclei 3 days after birth. Moreover, inactivation of the NT-3 gene in mice (Ernfors et al., 1994; Fariñas et al., 1994) leads to a 50% loss in neurons in the superior cervical ganglion. Thus, the normal expression of GDNF is not sufficient to maintain the sympathetic neurons in animals lacking NGF or NT-3 during development. It is possible that the small remaining population of neurons may represent GDNF-dependent cells or that GDNF affects a wider population of NGF- and NT-3-dependent neurons during other time windows of development (cf. Buchmann and Davies, 1993).

These complexities notwithstanding, we have demonstrated that GDNF has a stimulatory effect on sympathetic and ciliary ganglionic neurons. The data suggest the possibility that GDNF, or an endogenous ligand similar to GDNF, has potent neurotrophic functions supporting cell survival and neurite promotion in these neurons during stages of their development, with a spectrum of responsive neurons not shared by other known trophic factors. The present report also shows that sympathetic ganglion explants offer a robust and simple way to assay for the activity of GDNF, and possibly also for related molecules.

ACKNOWLEDGMENTS

We acknowledge the gift of recombinant purified GDNF from Synergen, Inc., Boulder, Colorado. We are grateful to Annika Kylberg for performing the bioassays and to Vibeke Nilsson for preparing the illustrations. This study was supported by the Swedish Natural Sciences and Medical Research Councils (grants B-AA/BU 04024-316 and B93-14X-03185-23B, respectively). B.J.H. was supported by a Visiting Scientist Award from the Swedish Medical Research Council (grant 14V 106 57).

REFERENCES

Attisano L, Wrana JL, Cheifetz S, Massagué J (1992): Novel activin receptors: Distinct genes and alternative mRNA splicing generate a repertoire of serine/threonine kinase receptors. Cell 68: 97–108.

Barnett JV, Moustakas A, Lin W, Wang X-F, Lin HY, Galper JB, Maas RL (1994): Cloning and developmental expression of the chick type II and type III TGFβ receptors. Dev Dyn 199:12–27.

Buchmann VL, Davies AM (1993): Different neurotrophins are expressed and act in a developmental sequence to promote the survival of embryonic sensory neurons. Development 118: 989–1001.

Burt DW (1992): Evolutionary grouping of the transforming growth

factor-β superfamily. Biochem Biophys Res Commun 184: 590–595.

Burt DW, Law AS (1994): Evolution of the transforming growth factor-beta superfamily. Prog Growth Factor Res 5:99–118.

Carri NG, Perris R, Johansson S, Ebendal T (1988): Differential outgrowth of retinal neurites on purified extracellular matrix molecules. J Neurosci Res 19:428–439.

Carri NG, Richarson P, Ebendal T (1994): Choroid coat extract and ciliary neurotrophic factor strongly promote neurite outgrowth in the embryonic chick retina. Int J Dev Neurosci 12:567–578.

Chalazonitis A, Kalberg J, Twardzik DR, Morrison RS, Kessler JA (1992): Transforming growth factor β has neurotrophic actions on sensory neurons in vitro and is synergistic with nerve growth factor. Dev Biol 152:121–132.

Crowley C, Spencer SD, Nishimura MC, Chen KS, Pitts-Meek S, Armanini MP, Ling LH, McMahon SB, Shelton DL, Levinson AD, Phillips HS (1994): Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. Cell 76:1001–1011.

Dagerlind Å, Friberg K, Bean A-J, Hökfelt T (1992): Sensitive mRNA detection using unfixed tissue: Combined radioactive and non-radioactive in situ hybridization histochemistry. Histochemistry 98:39–49.

Ebendal T (1987): Comparative screening for ciliary neurotrophic activity in organs of the rat and chicken. J Neurosci Res 17:19–24.

Ebendal T (1989): Use of collagen gels to bioassay nerve growth factor activity. In Rush RA (ed): "Nerve Growth Factors. IBRO Handbook Series: Methods in the Neurosciences." Vol 12. Chichester: John Wiley & Sons, pp 81–93.

Ebendal T, Jordell-Kylberg A, Söderström S (1978): Stimulation by tissue explants on nerve fibre outgrowth in culture. In Jacobson C-O, Ebendal T (eds): "Formshaping Movements in Neurogenesis." Zoon, Vol 6. Stockholm: Almqvist & Wiksell, pp 235–243.

Ebendal T, Olson L, Seiger Å, Hedlund K-O (1980): Nerve growth factors in the rat iris. Nature (London) 286:25–28.

Ebendal T, Olson L, Seiger Å, Belew M (1984): Nerve growth factors in chick and rat tissues. In Black IB (ed): "Cellular and Molecular Biology of Neuronal Development." New York: Plenum Press, pp 231–242.

Ebendal T, Strömberg I, Olson L, Seiger Å (1985): Parasympathetic neurotrophic activity in the rat iris: Determination after different denervations. Neuroscience 16:181–185.

Ernfors P, Ibáñez CF, Ebendal T, Olson L, Persson H (1990): Molecular cloning and neutrophic activities of a protein with structural similarities to nerve growth factor: Developmental and topographical expression in the brain. Proc Natl Acad Sci USA 87:5454–5458.

Ernfors P, Lee K-F, Kucera J, Jaenisch R (1994): Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. Cell 77:503–512.

Fariñas I, Jones KR, Backus C, Wang X-Y, Reichardt LF (1994): Severe sensory and sympathetic deficits in mice lacking neurotrophin-3. Nature 369:658–661.

Flanders KC, Lüdecke G, Engels S, Cissel DS, Roberts AB, Kondaiah P, Lafyatis R, Sporn MB, Unsicker K (1991): Localization and actions of transforming growth factor-βs in the embryonic nervous system. Development 113:183–191.

Hedlund K-O, Ebendal T (1978): Different ganglia from the chick embryo in studies on neuron development in culture. In Jacobson C-O, Ebendal T (eds): "Formshaping Movements in Neurogenesis." Zoon, Vol 6. Stockholm: Almqvist & Wiksell, pp 217–223.

Humpel C, Hoffer B, Strömberg I, Bektesh S, Collins F, Olson L (1994): Neurons of the hippocampal formation express glial cell line-derived neurotrophic factor messenger RNA in response to kainate-induced excitation. Neuroscience 59:791–795.

Kullander K, Ebendal T (1994): Neurotrophin-3 acquires NGF-like activity after exchange to five NGF amino acid residues: Molecular analysis of the sites in NGF mediating the specific interaction with gp140^{trk}. J Neurosci Res 39:195–210.

Lapthorn AJ, Harris DC, Littlejohn A, Lustbader JW, Canfiels RE, Machin KJ, Morgan FJ, Isaacs NW (1994): Crystal structure of human chorionic gonadotropin. Nature 369:455–461.

Lin HY, Lodish HF (1993): Receptors for the TGF-β superfamily: Multiple polypeptides and serine/threonine kinases. Trends Cell Biol. 3:14–19.

Lin HY, Wang X-F, Ng-Eaton E, Weinberg RA, Lodish HF (1992): Expression cloning of the TGF-β type II receptor, a functional transmembrane serine/threonine kinase. Cell 68:775–785.

Lin L-FH, Doherty DH, Lile JD, Bektesh S, Collins F (1993): GDNF: A glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 260:1130–1132.

Martinou J-C, Le Van Thai A, Valette A, Weber MJ (1990): Transforming growth factor β1 is a potent survival factor for rat embryo motoneurons in culture. Dev Brain Res 52:175–181.

Massagué J (1990): the transforming growth factor-β family. Annu Rev Cell Biol 6:597–641.

Massagué J (1992): Receptors for the TGFβ family. Cell 69:1067–1070.

Matthews LS, Vale WW (1991): Expression cloning of an activin receptor, a predicted transmembrane serine kinase. Cell 65: 973–982.

McDonald NQ, Hendrickson WA (1993): A structural superfamily of growth factors containing a cystine knot motif. Cell 73:421–424.

Schaar DG, Sieber B-A, Dreyfus CF, Black IB (1993): Regional and cell-specific expression of GDNF in rat brain. Exp Neurol 124: 368–371.

Schmidt-Kastner R, Tomac A, Hoffer B, Bektesh S, Rosenzweig B, Olson L (1994): Glial cell line-derived neurotrophic factor (GDNF) mRNA upregulation in striatum and cortical areas after pilocarpine-induced status epilepticus in rats. Mol Brain Res 26:325–330.

Schubert D, Heinemann S, Carlisle W, Tarikas H, Kimes B, Patrick J, Steinbach JH, Culp W, Brandt BL (1974): Clonal cell lines from the rat central nervous system. Nature 249:224–227.

Schubert D, Kimura H, LaCorbiere M, Vaughan J, Karr D, Fischer WH (1990): Activin is a nerve cell survival molecule. Nature 344:868–870.

Strömberg I, Björklund L, Johansson M, Tomac A, Collins F, Olson L, Hoffer B, Humpel C (1993): Glial cell line-derived neurotrophic factor is expressed in the developing but not adult striatum and stimulates developing dopamine neurons in vivo. Exp Neurol 124:401–412.

ten Dijke P, Yamashita H, Ichijo H, Franzén P, Laiho M, Miyazono K, Heldin C-H (1994): Characterization of type I receptors for transforming growth factor-β and activin. Science 264:101–104.

Williams R, Bäckström A, Ebendal T, Hallböök F (1993): Molecular cloning and cellular localization of trkC in the chicken embryo. Dev Brain Res 75:235–252.

Wrana JL, Attisano L, Weiser R, Ventura F, Massagué J (1994): Mechanism of activation of the TGF-β receptor. Nature 370: 341–347.

Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 5454–5458, July 1990
Biochemistry

Molecular cloning and neurotrophic activities of a protein with structural similarities to nerve growth factor: Developmental and topographical expression in the brain

(nerve growth factor family/cDNA/neurotrophic factor/hippocampal neurons/nerve growth factor receptor binding)

PATRIK ERNFORS*, CARLOS F. IBÁÑEZ*, TED EBENDAL†, LARS OLSON‡, AND HÅKAN PERSSON*§

*Department of Medical Chemistry, Laboratory of Molecular Neurobiology, and ‡Department of Histology and Neurobiology, Karolinska Institute, Box 60400, S-104 01 Stockholm, Sweden; and †Department of Developmental Biology, Biomedical Center, Uppsala University, S-751 23 Uppsala, Sweden

Communicated by Peter Reichard, April 25, 1990 (received for review April 2, 1990)

ABSTRACT **We have used a pool of degenerate oligonucleotides representing all possible codons in regions of homology between brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) to prime rat hippocampal cDNAs in the polymerase chain reaction. The amplified DNA included a product with significant similarity to NGF and BDNF, which was used to isolate a 1020-nucleotide-long cDNA from a rat hippocampal library. From the nucleotide sequence, a 282-amino-acid-long protein with ≈45% amino acid similarity to both pig BDNF and rat NGF was deduced. In the adult brain, the mRNA for this protein was predominantly expressed in hippocampus, where it was confined to a subset of pyramidal and granular neurons. The developmental expression in brain showed a clear peak shortly after birth, 1 and 2 weeks earlier than maximal expression of BDNF and NGF, respectively. It was also expressed in several peripheral tissues with the highest level in kidney. The protein, transiently expressed in COS cells, was tested on chicken embryonic neurons and readily stimulated fiber outgrowth from explanted Remak's ganglion and, to a lesser extent, the nodose ganglion. A weak, but consistent, fiber outgrowth response was also seen in the ciliary ganglion and in paravertebral sympathetic ganglia. Moreover, the protein displaced binding of NGF to its receptor, suggesting that it can interact with the NGF receptor. Thus, this factor, although structurally and functionally related to NGF and BDNF, has unique biological activities and represents a member of a family of neurotrophic factors that may cooperate to support the development and maintenance of the vertebrate nervous system.**

During development of the vertebrate nervous system, a vast overproduction of neurons is compensated for by naturally occurring neuronal death, which is regulated by their targets (1). Within the targets, specific proteins, referred to as neurotrophic factors, are produced in limiting amounts and the release of these proteins is believed to regulate both the timing and the extent of innervation (2).

In the peripheral nervous system, the most well-characterized neurotrophic factor, nerve growth factor (NGF), supports the development of sympathetic and neural crest-derived sensory neurons, and in the adult the maintenance of the sympathetic nervous system is critically dependent on NGF (3, 4). In agreement with a trophic role of NGF for adult sympathetic neurons, the levels of both NGF mRNA and protein correlate with the density of sympathetic innervation (5, 6). NGF mRNA and protein have also been found in the brain, with the highest levels in hippocampus and cerebral cortex, to which the major cholinergic pathways in the brain project (7–10). Basal forebrain cholinergic neurons can be prevented from dying after axonal transection by addition of NGF (11–15) and they respond to NGF *in vivo* by a marked increase in fiber outgrowth (16).

In addition to NGF, one other protein, termed brain-derived neurotrophic factor (BDNF), has been shown to be present in low amounts (17), secreted from cells (18), and to support survival of embryonic sensory neurons *in vivo* (19). In common with NGF, BDNF supports the survival of neural crest-derived embryonic sensory neurons *in vitro*, but nonoverlapping trophic activities are suggested by the finding that BDNF also supports placode-derived neurons from the nodose ganglia and retinal ganglion cells (20, 21), which are less sensitive to NGF (22, 23). Regulation of neuronal survival *in vivo* in the brain by BDNF has not yet been demonstrated, although its sites of synthesis have recently been mapped by *in situ* hybridization where a high level of labeling was found in hippocampal neurons (24).

NGF is synthesized as a preproprotein and the structure of both the precursor and the mature protein has been deduced from cDNA and genomic clones (25, 26). More recently, a genomic clone has been isolated for porcine BDNF (18). Of considerable interest is the finding that the mature BDNF and NGF proteins show striking amino acid similarities, suggesting that they are structurally related and may be members of a family of neurotrophic factors (18).

In this study, we report on the cloning and expression of an additional member of the NGF family.¶ Due to its restricted expression in the brain, being mostly confined to a subset of pyramidal and granular neurons in the hippocampus, we have named this protein hippocampus-derived neurotrophic factor (HDNF).

MATERIALS AND METHODS

RNA Preparation, Molecular Cloning, and DNA Sequencing. Polyadenylylated RNA [poly(A)$^+$] was prepared as described (27). For cloning, rat hippocampus poly(A)$^+$ RNA (5 μg) was used as a template for synthesis of single-stranded cDNA using Moloney murine leukemia virus reverse transcriptase (Pharmacia). Six separate mixtures of 28-mer oligonucleotides representing all possible codons corresponding to the amino acid sequence KQYFYET (5′-oligonucleotide) and WRFIRID (3′-oligonucleotide) were synthesized on an Applied Biosystems A381 DNA synthesizer. The 5′-oligonucleotide contained a synthetic *Eco*RI site and the 3′-oligonucleotide contained a synthetic *Hin*dIII site. Each mixture of oligonucleotides was then used to prime the amplification of hippocampal cDNA (25 ng) by the polymer-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; HDNF, hippocampus-derived neurotrophic factor; PCR, polymerase chain reaction.

§To whom reprint requests should be addressed.

¶The sequence reported in this paper has been deposited in the GenBank data base (accession no. M34643).

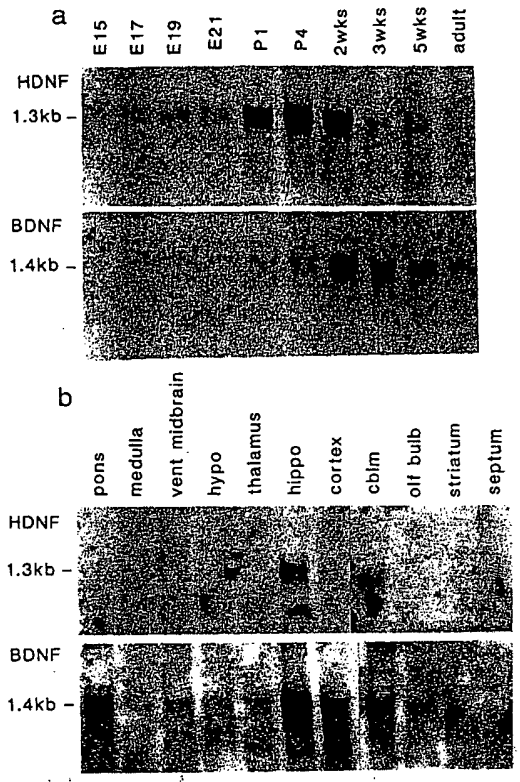


FIG. 2. Developmental and regional expression of HDNF and BDNF mRNA in rat brain. (*a*) Poly(A)$^+$ RNA (20 μg per slot) isolated from Sprague–Dawley rat brain at the indicated developmental stages was hybridized to the indicated probes (HDNF and BDNF). Adult rats were 12 weeks old. E, embryonic day; P, postnatal day; wks, weeks. (*b*) Same analysis as in *a* using poly(A)$^+$ RNA (20 μg per slot) isolated from the indicated regions of adult male Sprague–Dawley rat brain. Medulla, medulla oblongata; hypo, hypothalamus; hippo, hippocampus; cortex, cerebral cortex; cblm, cerebellum; olf, olfactory bulb.

brain showed remarkable regional specificity with high levels in hippocampus compared with other brain regions analyzed (Fig. 2*b*). In fact, cerebellum was the only other region where HDNF mRNA was clearly detected, with the exception of cerebral cortex, which showed a weak signal. BDNF mRNA was more widely distributed in rat brain, although hippocampus also contained the highest amount, followed by cerebral cortex, pons, and cerebellum (Fig. 2*b*).

Neurons Expressing HDNF and BDNF mRNA Are Located in a Distinct Topographical Arrangement in Hippocampus. Anterior sections of the dorsal hippocampus showed neurons expressing high levels of HDNF mRNA primarily confined to the medial part of CA1 and CA2 (Fig. 3 *a* and *c*). Few HDNF mRNA-expressing neurons were also found in lateral parts of CA1. Granular cells of the dentate gyrus were also highly labeled (Fig. 3*a*). CA3 and hilar cells of the dentate gyrus showed no labeling for HDNF mRNA at any level (Fig. 3*d*). No labeling was seen over any sections after hybridization to a control probe, complementary to the specific HDNF probe. Adjacent sections hybridized to a BDNF-specific probe revealed labeling over granular neurons in the dentate gyrus (Fig. 3*b*), although possibly with lower intensity than that seen after hybridization for HDNF mRNA. Strong labeling with the BDNF-specific probe was found over neurons in the hilar region (Fig. 3*e*), CA3, and part of CA2 (Fig. 3*b*). Few BDNF mRNA-expressing neurons, which appeared to be less intensively labeled, were also detected in CA1 and CA2 (Fig. 3*b*). Intensely labeled neurons were seen in claustrum, located lateral to the external capsule. This region showed no labeling for HDNF mRNA.

Neurotrophic Activities of HDNF in Explanted Chicken Embryonic Ganglia. The 1020-bp HDNF cDNA insert was cloned in the expression vector pXM (34), designed for transient expression in COS cells. Two plasmid constructs were isolated, containing the HDNF insert either in the correct or opposite orientation for translation of the HDNF protein. The latter construct was used as a negative control. Included was also a construct containing the rat NGF gene (36). The different constructs were transfected into COS cells and 3 days later conditioned medium was tested for biological activity in bioassays that measured fiber outgrowth from various chicken embryo ganglia. A marked stimulation of neurite outgrowth, consistently resulting in circular or oval fiber halos, was seen in the ganglion of Remak, a ganglionated nerve trunk in the mesorectum of the chicken embryo (38, 39) (Fig. 4*a*). Although NGF is known to stimulate the explanted ganglion of Remak (39), it was far less efficient than HDNF (Fig. 4*b*). A modest stimulation of fiber outgrowth was also seen with HDNF in the nodose ganglion, consisting of neurons exclusively derived from an epidermal placode (22)

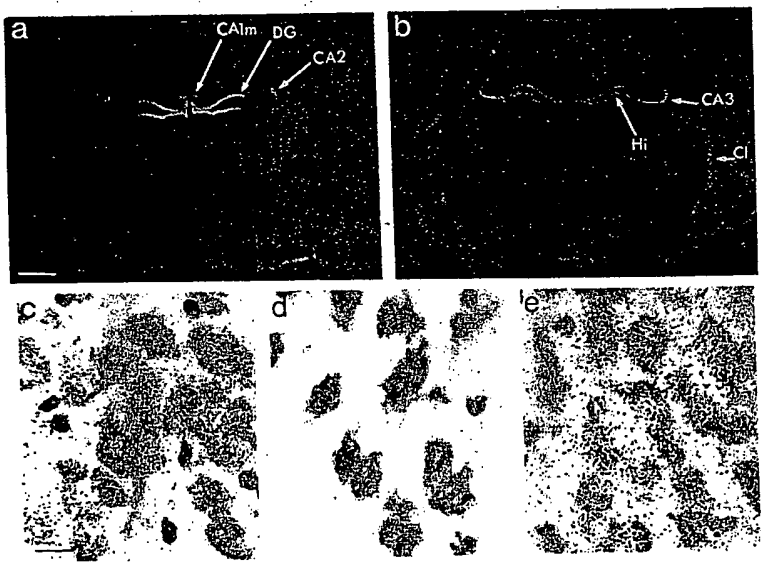


FIG. 3. Expression of HDNF and BDNF mRNA in hippocampal neurons. Rat (Sprague–Dawley) brain sections hybridized to either HDNF- or BDNF-specific oligonucleotide probes. (*a*) Autoradiogram from a section at the level of hippocampus hybridized to the HDNF-specific probe. Note labeling over medial CA1, CA2, and the dentate gyrus. (*b*) Adjacent section hybridized to a BDNF-specific probe. Note labeling over CA2 and CA3 as well as hilar cells and dentate granule layer. (*c*) Pyramidal neurons in medial CA1 labeled with the HDNF-specific probe. (*d*) Nonlabeled hilar neurons after hybridization to the HDNF-specific probe. (*e*) Hilar neurons labeled with the BDNF-specific probe. DG, dentate gyrus; CA1m, CA1 medial; Hi, hilus of dentate gyrus; Cl, claustrum. (*a* and *b*, bar = 1.3 mm; *c–e*, bar = 10 μm.)

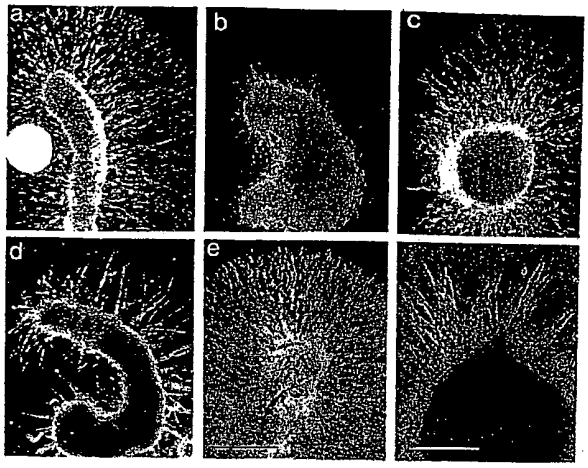


FIG. 4. Stimulation of fiber outgrowth from chicken embryonic ganglia. Biological activity of recombinant HDNF shown as effects on different nerve tissues from the chicken embryo. Remak ganglion stimulated by HDNF (*a*) or NGF (*b*). (*c*) Nodose ganglion with HDNF. Paravertebral sympathetic ganglion in response to HDNF (*d*) and recombinant rat NGF (*e*). (*f*) Ciliary ganglion with HDNF. All figures show ganglia after 1.5 days in culture. Dark-field microscopy. (Bars = 0.3 mm.)

(Fig. 4*c*). Again, HDNF was superior to NGF in evoking this response. A weak, but consistent, fiber outgrowth response with HDNF was seen in paravertebral sympathetic trunk ganglia (Fig. 4*d*), which, however, was much less pronounced compared with the massive response to rat NGF (Fig. 4*e*). In the ciliary ganglion, a weak but consistent fiber outgrowth response, manifested by the projection of short neurite fascicles, was seen with HDNF but never with NGF (Fig. 4*c*). In the dorsal root ganglia, HDNF stimulated neurite outgrowth to the same extent as NGF.

Displacement of NGF Binding to PC12 Cells by HDNF. Concentrated conditioned medium from transfected COS cells was tested for its ability to compete for binding ^{125}I-labeled NGF (^{125}I-NGF) to its receptor on PC12 cells. The concentration of ^{125}I-NGF used allowed ≈80% of the labeled NGF to be bound to the low-affinity receptor site in the absence of competition (40). Twenty-five times concentrated medium containing the HDNF protein displaced ≈70% of the labeled NGF and a 20% displacement was seen after a 25-fold dilution (Fig. 5). In contrast, 25 times concentrated medium from COS cells transfected with the HDNF cDNA in the opposite orientation did not show any displacement. Concentrated medium from cells transfected in parallel with a rat NGF gene displaced 50% of the labeled NGF when diluted 250 times.

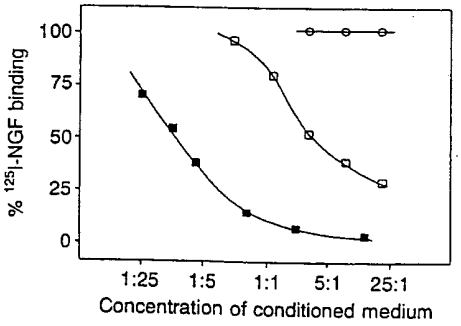


FIG. 5. Displacement of ^{125}I-NGF from its receptor on PC12 cells by HDNF and NGF. Serial dilutions of transfected COS cell medium with (□) or without (○) HDNF or containing rat NGF (■) were analyzed for their ability to displace ^{125}I-NGF from its receptor on PC12 cells. Data are from two independent experiments that showed a variation of ±20%.

DISCUSSION

The cDNA clone isolated in this study encodes a protein, HDNF, with a remarkable sequence similarity to both NGF and BDNF and therefore represents an additional member of a family of neurotrophic proteins. Recently (at the time of submission of this manuscript), two groups (41, 42) independent of us isolated genomic clones for a protein (neurotrophin 3) from mouse and rat, respectively, which is identical to the neurotrophic protein characterized in this study. Our cDNA clone predicts a 282-amino-acid-long protein, which is 24 amino acids longer than the protein deduced from the genomic clones (41, 42). Two alternative start sites for translation of the NGF protein have been proposed; the first is located in a separate 5′ exon (43). The second start site, located in the 3′ exon, is also efficiently used for translation of the NGF protein (36, 44) and generates a 68-amino acid shorter protein. Thus, the structure of our cDNA clone indicates that the HDNF protein utilizes two alternative start sites for translation, located in separate exons, and suggests that the genomic organization of HDNF and NGF is very similar.

In peripheral ganglia bioassays, HDNF showed neurotrophic activities that were to some extent reminiscent of both NGF and BDNF. Thus, in similarity to BDNF (20), HDNF stimulated fiber outgrowth from the nodose ganglia and, as for NGF, evoked a fiber outgrowth response in sympathetic ganglia. In the latter case, however, the response was clearly weaker than with NGF. The partially overlapping activities seen *in vitro* may reflect a cooperation of these factors *in vivo*, where two or more proteins from the same family may support the development and/or maintenance of specific neurons. The most striking stimulation of fiber outgrowth evoked by HDNF was seen in the peripheral, autonomic, ganglion of Remak containing mostly cholinergic but also some adrenergic neurons (38, 39). This effect was clearly more pronounced than effects seen with NGF (39), suggesting that HDNF also evokes trophic responses different from both NGF and BDNF. In agreement with this, HDNF showed a weak, but consistent, neurite outgrowth response in the ciliary ganglion, which does not respond to NGF or BDNF. The ciliary ganglion is known to respond to ciliary neurotrophic factor (45), which lacks a signal sequence, but could be released by an as yet unknown mechanism (46). Thus, HDNF is the only secreted neurotrophic factor today that is known to affect fiber outgrowth, at least *in vitro*, from the ciliary ganglion.

The HDNF protein displaced ^{125}I-NGF from PC12 cells, indicating that it can interact with the NGF receptor. With the assumption that NGF and HDNF were produced in equal amounts in parallel transfections and that the conditioned medium lacks interfering substances, the interaction of NGF to its receptor was 30-fold more efficient. PC12 cells have both low- and high-affinity receptors but only the high-affinity receptor mediates a biological response (47). The fact that recombinant rat NGF readily stimulated neurite outgrowth from PC12 cells, whereas HDNF, even at 30-fold higher concentrations than NGF, did not suggests that HDNF can only interact with the NGF receptor in its low-affinity form. It therefore appears likely that the biological responses elicited by HDNF are mediated by either a separate second messenger system compared with NGF or that the HDNF receptor is different from the NGF receptor.

In similarity with NGF, HDNF mRNA was found in several peripheral rat tissues, with the highest level in kidney. Hybridization of the same filters to a rat NGF probe revealed that the level of HDNF mRNA in kidney was only slightly

higher than the levels of NGF mRNA in peripheral sympathetic target tissues, indicating that HDNF is produced in relatively small amounts in peripheral rat tissues. This is also true for the brain, and the fact that seven positive cDNA clones were isolated from 1.2×10^6 independent clones tests that in hippocampus, containing the highest level of NF mRNA, this transcript constitutes ≈1 in every 170,000, which clearly represents a rare transcript. Thus, as in the case of NGF, HDNF may be present in limiting amounts and functions *in vivo* as a target-derived factor for a specific subset of both peripheral and central neurons. The regional distribution of HDNF mRNA in the periphery is, however, different from NGF, and, in agreement with the *in vitro* biological assays, HDNF may support a different set of peripheral neurons. Of interest is also that HDNF mRNA was found in the ovary, whereas no mRNA was detected in the testis, where both NGF and its receptor is expressed (48) and where NGF has been suggested to mediate an interaction between Sertoli cells and germ cells (49). This shows that different members of the NGF family are expressed in different reproductive tissues and suggests that they may have nonoverlapping functions outside the nervous system.

Interestingly, the three neurotrophic proteins were maximally expressed at different times of brain development with a peak of HDNF mRNA shortly after birth, BDNF mRNA around 2 weeks, and NGF mRNA around 3 weeks after birth (see ref. 8 for NGF). Moreover, the mRNA's for all three proteins were expressed in hippocampus at levels higher than in other regions, particularly in the case of HDNF. Within hippocampus, all three mRNAs were also confined to neurons (see ref. 10 for NGF) and a clear topographical division was seen, where HDNF mRNA was concentrated to pyramidal neurons in medial CA1, CA2, and granular neurons in dentate gyrus. Strongly labeled BDNF neurons were primarily seen in CA3 and the hilar region of dentate gyrus. Neurons h apparent lower levels of BDNF mRNA were seen in the tate gyrus. The hilar region, containing neurons with high levels of BDNF mRNA, showed no labeling for HDNF mRNA.

This remarkable concentration of trophic factors in the adult hippocampus suggests that maintenance of plasticity is crucial to its function and may relate to the presumed morphological sequelae of long-term potentiation and memory consolidation processes. The intriguing temporal and spatial expression of the three neurotrophic proteins in the brain suggests that they predominantly support neuronal innervation at different times of development and that they may also exert specific trophic support for different central nervous system neurons, a possibility that will be an interesting topic for future studies.

We thank Annika Jordell-Kylberg for expert technical assistance with the bioassays. This work was supported by The Swedish Natural Science Research Council, the Swedish Medical Research Council, the Swedish Natural Environment Protection Board, Magnus Bergvalls Stiftelse, US Grants (University of Colorado) AG 04418 and NS-09199, and funds from the Karolinska Institute. P.E. was supported by the Swedish Medical Research Council.

1. Cowan, W. M., Fawcett, J. W., O'Leary, D. D. M. & Stanfield, B. B. (1984) *Science* 225, 1258–1265.
2. Black, I. B. (1986) *Proc. Natl. Acad. Sci. USA* 83, 8249–8252.
3. Levi-Montalcini, R. (1987) *Science* 237, 1154–1162.
4. Thoenen, H. & Barde, Y.-A. (1980) *Physiol. Rev.* 60, 1284–1335.
5. Heumann, R., Korsching, S., Scott, J. & Thoenen, H. (1984) *EMBO J.* 3, 3183–3189.
6. Shelton, D. L. & Reichardt, L. F. (1984) *Proc. Natl. Acad. Sci. USA* 81, 7951–7955.
7. Korsching, S., Auberger, G., Heumann, R., Scott, J. & Thoenen, H. (1985) *EMBO J.* 4, 1389–1393.
8. Whittemore, S. R., Ebendal, T., Lärkfors, L., Olson, L., Seiger, Å., Strömberg, I. & Persson, H. (1986) *Proc. Natl. Acad. Sci. USA* 83, 817–821.
9. Shelton, D. L. & Reichardt, L. F. (1986) *Proc. Natl. Acad. Sci. USA* 83, 2714–2718.
10. Ayer-LeLièvre, C., Olson, L., Ebendal, T., Seiger, Å. & Persson, H. (1988) *Science* 240, 1339–1341.
11. Hefti, F. (1986) *J. Neurosci.* 6, 2155–2162.
12. Williams, L. R., Varon, S., Peterson, G. M., Wictorin, K., Fischer, W., Björklund, A. & Gage, F. H. (1986) *Proc. Natl. Acad. Sci. USA* 83, 9231–9235.
13. Kromer, L. F. (1987) *Science* 235, 214–216.
14. Rosenberg, M. B., Friedmann, T., Robertson, R. C., Tuszynski, M., Wolff, J. A., Breakefield, X. O. & Gage, F. (1988) *Science* 242, 1575–1578.
15. Strömberg, I., Wetmore, C. J., Ebendal, T., Ernfors, P., Persson, H. & Olson, L. (1990) *J. Neurosci. Res.* 25, 405–411.
16. Ernfors, P., Ebendal, T., Olson, L., Mouton, P., Strömberg, I. & Persson, H. (1989) *Proc. Natl. Acad. Sci. USA* 86, 4756–4760.
17. Barde, Y.-A., Edgar, D. & Thoenen, H. (1982) *EMBO J.* 1, 549–553.
18. Leibrock, J., Lottspeich, F., Hohn, A., Hofer, M., Hengerer, B., Masiakowski, P., Thoenen, H. & Barde, Y.-A. (1989) *Nature (London)* 341, 149–152.
19. Hofer, M. M. & Barde, Y.-A. (1988) *Nature (London)* 331, 261–262.
20. Lindsay, R. M., Thoenen, H. & Barde, Y.-A. (1985) *Dev. Biol.* 112, 319–328.
21. Johnson, J. E., Barde, Y.-A., Schwab, M. & Thoenen, H. (1986) *J. Neurosci.* 6, 3031–3038.
22. Hedlund, K. & Ebendal, T. (1980) *J. Neurocytol.* 9, 665–682.
23. Davies, A. M., Thoenen, H. & Barde, Y.-A. (1986) *J. Neurosci.* 6, 1897–1904.
24. Wetmore, C., Ernfors, P., Persson, H. & Olson, L. (1990) *Exp. Neurol.* 109, in press.
25. Scott, J., Selby, M., Urdea, M., Quiroga, M., Bell, G. I. & Rutter, W. J. (1983) *Nature (London)* 302, 538–540.
26. Ullrich, A., Gray, A., Berman, C. & Dull, T. J. (1983) *Nature (London)* 303, 821–825.
27. Ernfors, P., Hallböök, F., Ebendal, T., Shooter, E. M., Radeke, M. J., Misko, T. P. & Persson, H. (1988) *Neuron* 1, 983–996.
28. Sanger, F., Nicklen, S. & Coulson, A. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463–5467.
29. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
30. Frohman, M. A., Dush, M. K. & Martin, G. P. (1988) *Proc. Natl. Acad. Sci. USA* 85, 8998–9002.
31. Whittemore, S. R., Friedman, P. L., Larhammar, D., Persson, H., Gonzalez-Carvajal, M. & Holets, V. R. (1988) *J. Neurosci. Res.* 20, 403–410.
32. Minty, A. J., Caravatti, M., Benoit, R., Cohen, A., Daubas, P., Weydert, A., Gros, F. & Buckingham, M. E. (1981) *J. Biol. Chem.* 286, 1008–1014.
33. Ernfors, P., Henschen, A., Olson, L. & Persson, H. (1989) *Neuron* 2, 1605–1613.
34. Yang, Y. C., Ciarlette, A. B., Temple, P. A., Chung, M. P., Kovacic, S., Wilek-Giannotti, J. S., Leary, N. C., Kriz, R., Donahue, R. E., Wong, G. G. & Clark, S. C. (1986) *Cell* 47, 3–10.
35. Luthman, H. & Magnusson, G. (1983) *Nucleic Acids Res.* 11, 1295–1305.
36. Ibanez, C., Hallböök, F., Ebendal, T. & Persson, H. (1990) *EMBO J.* 9, 1477–1483.
37. Ebendal, T. (1989) *IBRO Handb. Ser.* 12, 81–93.
38. Le Douarin, N. M., Teillet, M. A., Ziller, C. & Smith, J. (1978) *Proc. Natl. Acad. Sci. USA* 75, 2030–2034.
39. Ebendal, T. (1979) *Dev. Biol.* 72, 276–290.
40. Sutter, A., Riopelle, R. J., Harris-Warrick, R. M. & Shooter, D. M. (1979) *J. Biol. Chem.* 254, 5972–5982.
41. Hohn, A., Leibrock, J., Bailey, K. & Barde, Y.-A. (1990) *Nature (London)* 344, 339–341.
42. Mainsonpierre, P. C., Belluscio, L., Squinto, S., Ip, N. Y., Furth, M. E., Lindsay, R. M. & Yancopoulos, G. D. (1990) *Science* 247, 1446–1451.
43. Selby, M. J., Edwards, R., Sharp, F. & Rutter, W. J. (1987) *Mol. Cell Biol.* 7, 3057–3064.
44. Hallböök, F., Ebendal, T. & Persson, H. (1988) *Mol. Cell. Biol.* 8, 452–456.
45. Adler, R., Landa, K., Manithorpe, M. & Varon, S. (1979) *Science* 204, 1434–1436.
46. Stöckli, K. A., Lottspeich, F., Sendtner, M., Maslakowski, P., Carroll, P., Götz, R., Lindholm, D. & Thoenen, H. (1989) *Nature (London)* 342, 920–923.
47. Eveleth, D. D. & Bradshaw, R. A. (1988) *Neuron* 1, 929–936.
48. Ayer-LeLiévre, C., Olson, L., Ebendal, T., Hallböök, F. & Persson, H. (1988) *Proc. Natl. Acad. Sci. USA* 85, 2628–2632.
49. Persson, H., Ayer-LeLiévre, C., Söder, O., Villar, M. J., Metsis, M., Olson, L., Ritzen, M. & Hökfelt, T. (1990) *Science* 247, 704–707.

F

SYNOPSIS OF APPLICATION OF WRITTEN DESCRIPTION GUIDELINES

Contents

SYNOPSIS OF APPLICATION OF WRITTEN DESCRIPTION GUIDELINES

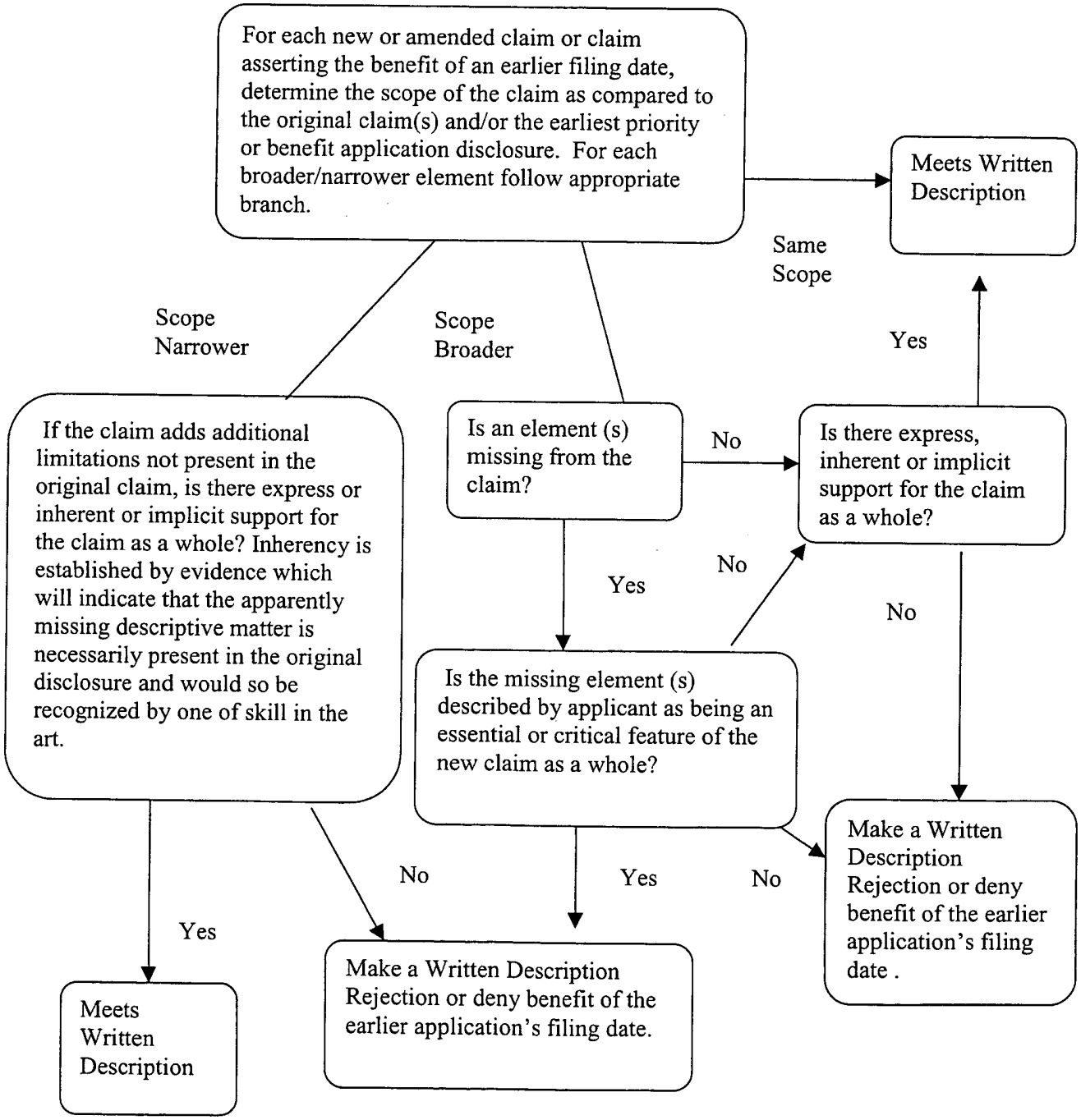
It is assumed at this point in the analysis that the specification has been reviewed and an appropriate search of the claimed subject matter has been conducted. It is also assumed that the examiner has identified which features of the claimed invention are conventional taking into account the body of existing prior art. There is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed. If the examiner determines that the application does not comply with the written description requirement, the examiner has the initial burden, after a thorough reading and evaluation of the content of the application, of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims. It should also be noted that the test for an adequate written description is separate and distinct from the test under the enablement criteria of 35 U.S.C. § 112 first paragraph. The absence of definitions or details for well-established terms or procedures should not be the basis of a rejection under 35 U.S.C. 112, para. 1, for lack of adequate written description. Limitations may not, however, be imported into the claims from the specification.

The following examples only describe how to determine whether the written description requirement of 35 U.S.C. 112, para. 1 is satisfied. Regardless of

the outcome of that determination, Office personnel must complete the patentability determination under all the relevant statutory provisions of Title 35 of the U.S. Code. Once Office personnel have concluded analysis of the claimed invention under all the statutory provisions, including 35 U.S.C. 101, 112, 102, and 103, they should review all the proposed rejections and their bases to confirm their correctness. Only then should any rejection be imposed in an Office action. The Office action should clearly communicate the findings, conclusions, and reasons which support them. When possible, the Office action should offer helpful suggestions on how to overcome rejections.

Written Description Amended or New Claims, or Claims Asserting the Benefit of an Earlier Filing Date

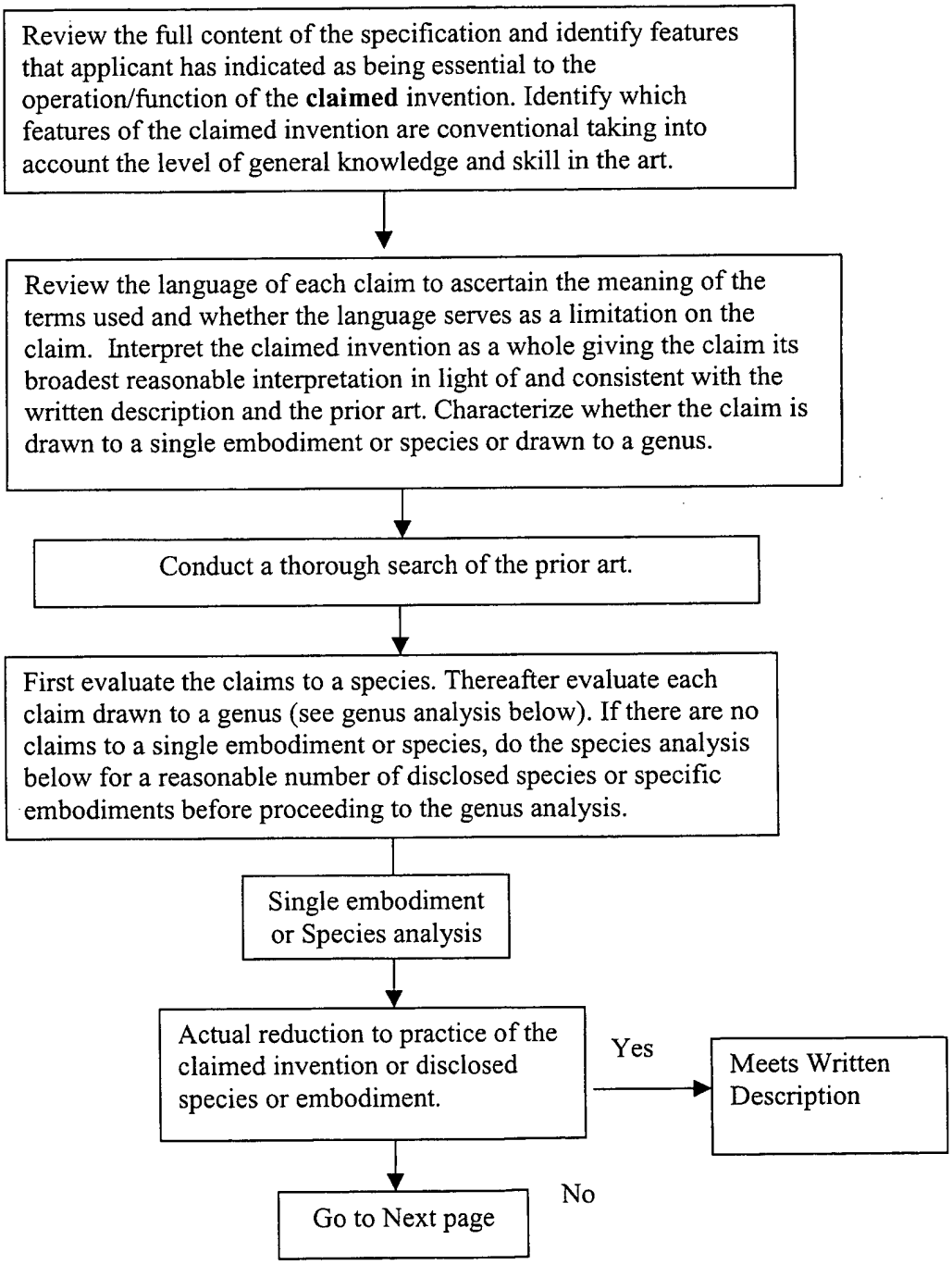
Decision Tree



Written Description

Original Claims

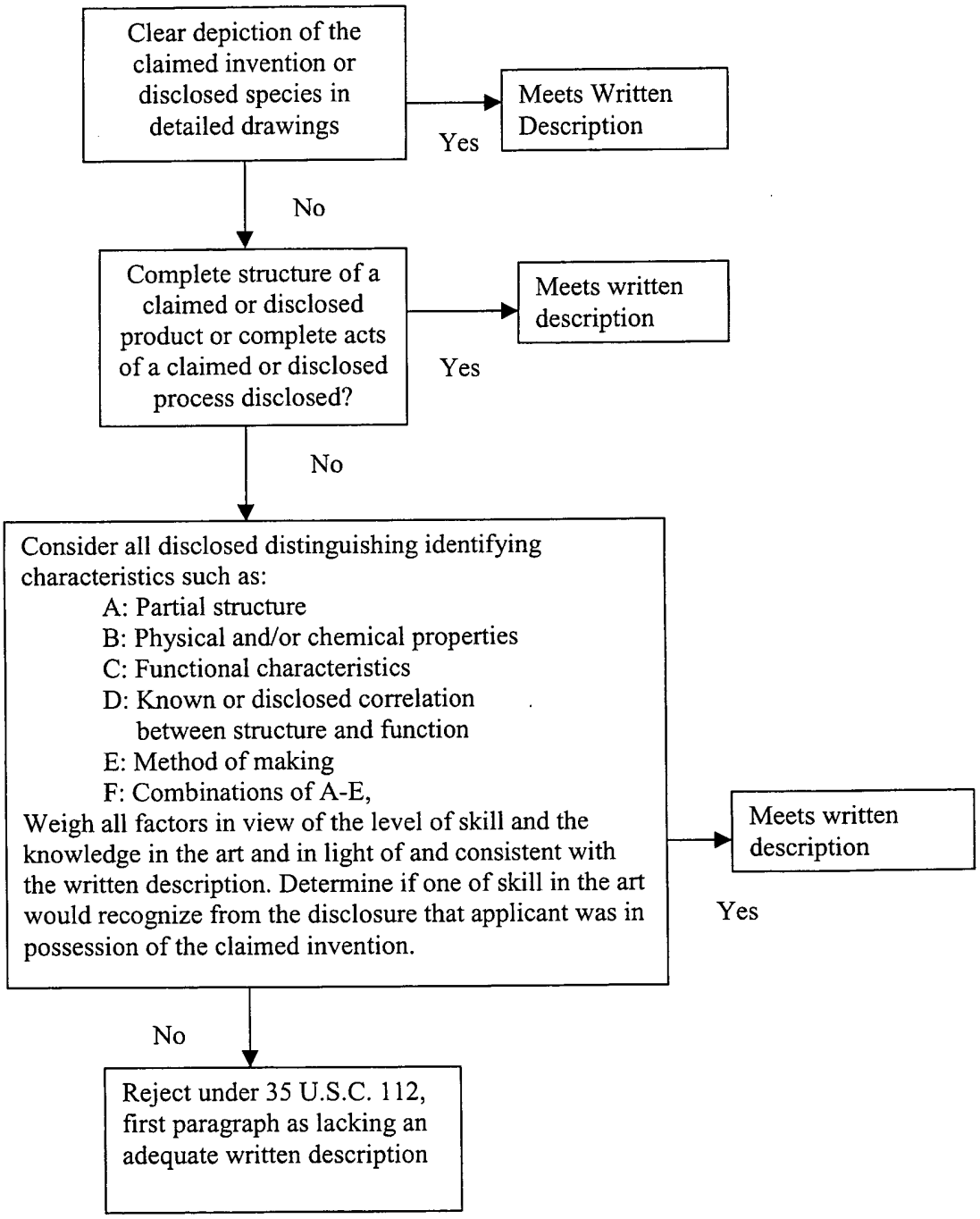
--Decision Tree--



Written Description

Original Claims

--Decision Tree--



Written Description

Original Claims

Decision Tree

--Page 3--

Genus Analysis

Determine whether the art indicates substantial variation among the species within the genus of the claimed subject matter.

Is there is a representative number of species implicitly or explicitly disclosed? What is a representative number of species depends on whether one of skill in the art would recognize that applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed or claimed.

Yes → **Meets Written Description**

No → Make a rejection under 35 USC 112 first paragraph as lacking written description.

WRITTEN DESCRIPTION TRAINING EXAMPLES

Example 1: Amended claims

Fact Pattern:

The specification is directed to a sectional sofa with a console between two reclining chairs, wherein control means for the reclining chairs are mounted on the console. The original disclosure clearly identifies the console as the only possible location for the controls, and provides for only the most minor variation in the location of the controls, e.g., the controls may be mounted on the top or side surfaces of the console or on the front wall. Additionally, the specification states that the purpose for the console is to house the controls. The original claims required the control elements to be present in the console. Applicant subsequently amends the claims to remove this limitation.

Amended Claim:

1. (Amended) A sectional sofa comprising:

a pair of reclining seats disposed in parallel relationship with one another in a double reclining seat sofa section, said double reclining seat sofa section being without an arm at one end whereby a second sofa section of the sectional sofa can be placed in abutting relationship with the end of the double reclining seat sofa section without an arm so as to form a continuation thereof,

each of said reclining seats having a backrest and seat cushion and movable between upright and reclined positions, said backrests and seat cushions of the pair of reclining sets lying in respective common planes when the seats are in the same positions,

a fixed console disposed in the double reclining seat sofa section between the pair of reclining seats and with the console and reclining seats together comprising a unitary structure, said console including an armrest portion for each of the reclining seats, said arm rests remaining fixed when the reclining seats move from one to another of their positions, and

a pair of control means [located upon the center console to enable each of the pair of reclining seats to move separately between the reclined and upright positions] mounted on the double reclining seat sofa section and each readily accessible to an occupant of its respective reclining seat and when actuated causing the respective reclining seat to move from the upright to the reclined position.

Analysis:

The amended claim is broader than the original claim in that the pair of control means is no longer required to be located on the center console. Thus, control means mounted on a center console is an element missing from the claim. The specification describes the location of the control means on the console as an essential feature of the claimed invention as a whole because the specification clearly identifies the console as the only possible location for the controls, and states that the purpose for the console is to house the controls.

Conclusion:

Reject the amended claim under 35 USC §112 first paragraph as lacking adequate written description.

Example 2: 35 USC 120 Priority

Fact Pattern:

The specification is directed to artificial hip sockets that include cup implants adapted for insertion into an acetabular, or hip, bone. The specification indicates that the shape of the cup is not important, as long as the implant can effectively function as an artificial hip socket. The application is a continuation in part of a parent application that describes an acetabular cup prosthesis wherein the cup is a trapezoid, a truncated cone, or of conical shape. All of these terms describe a conical cup. The parent specification also touts the criticality of a conical cup over all other shape cups.

A reference disclosing the claimed invention published between the filing date of the parent application and the instant application. Applicant asserts entitlement to the filing date of the parent application.

Claim:

1. An acetabular cup prosthesis comprising (1) a body extending generally longitudinally and terminating into front and rear surfaces, said front surface extending substantially transversely to said body; and (2) at least one fin for securing said cup to a prepared acetabulum cavity, said fin having a length extending generally longitudinally from said front surface toward said rear surface continuously along said body throughout the entire length of said fin, and said fin being configured so as to extend radially outwardly beyond the perimeter of said front surface and said body so as to engage with the cavity thereby securing said cup.

2. The prosthesis of claim 1, wherein the body has a generally conical outer surface.

Analysis:

Claim 1 in the instant application is directed to an acetabular cup prosthesis wherein the shape of the cup is not specifically defined (see element (1) of claim 1). The claim is broader than the disclosure in the parent application, which only describes a conical cup. Claim 1 is missing the element of a conical shape. This element is an essential or critical feature of the invention described in the parent application because the parent application only discloses a conical shape and the conical shape is described as critical over other shapes.

Claim 2 of the instant application is directed to an acetabular cup prosthesis wherein the cup has a generally conical outer surface. The claim is of the same scope as the invention described in the parent application.

Conclusion:

Reject claim 1 over the prior art reference, and indicate that the claim is not entitled to the benefit of the earlier application filing date.

Indicate that claim 2 is entitled to the benefit of the parent application filing date.

Note that if applicant had added the subject matter of claim 1 of this application to the parent application in an amendment, the claim would have been rejected under 35 U.S.C. 112, first paragraph as lacking an adequate written description.

Example 2A: Essential element missing from original claim

Fact Pattern:

The fact situation of example 2 above is similar to the fact situation of the instant example, however, there is no parent application in this example.

The specification is directed to artificial hip sockets that include cup implants adapted for insertion into an acetabular, or hip, bone. The specification indicates that the shape of the cup is critical to permit the implant to effectively function as an artificial hip socket. The application describes an acetabular cup prosthesis wherein the cup is a trapezoid, a truncated cone, or of conical shape. All of these terms describe a conical cup. The specification also touts the criticality of a conical cup.

Claims: Same as claims 1 and 2 of example 2 above.

Analysis:

Claim 1 in the instant application is directed to an acetabular cup prosthesis wherein the shape of the cup is not specifically defined (see element (1) of claim 1). The claim is broader than the disclosure in the instant application that only describes a conical cup. Claim 1 is missing the element of a conical shape. A review of the specification indicates that a cup implant having a shape which can effectively function as an artifical hip socket is critical to the operation/function of the claimed invention. The application discloses a conical shape cup and the conical shape is described as critical over other shapes. The specification indicates that the invention **as claimed** will not function in its intended manner without the specific cup

shape. Therefore this element is essential to the function/operation of the invention.

Claim 1 is directed to a genus. There is no actual reduction to practice or clear depiction of the claimed invention in detailed drawings; however, the complete structure of a species of the claimed prosthesis (with conical shape) is disclosed. The disclosed species is not representative of the genus because the specification indicates that without the conical shape the invention will not operate as intended. Therefore, applicant was not in possession of the necessary common attributes of the elements possessed by the members of the genus. A written description rejection should be made in this situation.

Example 2B: A preferred element missing from original claim

Fact Pattern:

The fact situation of example 2B is similar to example 2A above except that in this example the shape of the conical cup is described as being preferred.

The specification is directed to artificial hip sockets that include cup implants adapted for insertion into an acetabular, or hip, bone. The specification indicates that the shape of the cup must permit the implant to effectively function as an artificial hip socket. The application describes an acetabular cup prosthesis wherein the cup is preferably a trapezoid, a truncated cone, or of conical shape. All of these terms describe a conical cup. The specification emphasizes that a conical cup is the preferred embodiment.

Claims: Same as claims 1 and 2 of example 2 above.

Analysis:

Claim 1 in the instant application is directed to an acetabular cup prosthesis wherein the shape of the cup is not specifically defined (see element (1) of claim 1). The claim is broader than the disclosure in the instant application that only describes a conical cup. Claim 1 is missing the element of a conical shape. A review of the specification indicates that a cup implant having a conical shape is preferred but has no apparent bearing to the operation/function of the claimed invention. Therefore this element is not essential to the function or operation of the invention.

Claim 1 is directed to a genus. Although there is no actual reduction to practice or clear depiction of the claimed invention in detailed drawings, the complete structure of a species of the claimed prosthesis (with conical shape) is disclosed. The disclosed species is representative of the genus because there is a known correlation between the structure and the function of claimed invention and one of skill in the art would recognize that applicant was in possession of the necessary common attributes of the elements possessed by the members of the genus. The invention as claimed will function in its intended manner even without the specific cup shape. No written description rejection should be made in this situation.

Note: ***If the specification needs to be amended to be consistent with an original claim, see MPEP 608.01(o).***

Example 3: New claims

Fact Pattern:

The specification describes a form of computer technology called multi-threading. In essence, computers with multi-threading capabilities can switch between tasks with such rapidity that they appear to be performing two or more tasks at once. The specification describes one illustrative example in the specification wherein one of the program threads is an editor and another thread is a code processing routine in the form of a compiler. As the operator strikes keys at the keyboard, the compiler thread executes between each successive pair of keystrokes to process the entered source code concurrently with the editing operation. By the time the operator has finished entering or editing the code the compiler thread will have completed most of the required processing, thereby freeing the operator from lengthy periods of waiting for extensive code processing.

In this illustrative embodiment the interrupt operation of the central processor is periodically activated by a timer or clock. Each interrupt operation asynchronously preempts the executing compiler thread and passes control of the central processor to an interrupt service routine. The input port is then polled to test if a key has been struck at the keyboard. If not, the interrupt is terminated and control returns to the compiler thread. If polling the port reveals that a key has been struck then the interrupt service routine invokes the editor thread which takes control of the central processor to perform a character code entry or other edit operation. In addition to the description above, the application's abstract references an editor, compiler, interrupt means, and return means, and the "Object of the Invention" section

and the "Description of Prior Art" clearly discuss the importance of an editor and compiler.

The original claims required, *inter alia*, an editor, a compiler, an interrupt means and a return means. These elements are missing from new claim 20.

Claim:

20. A computer-readable disk memory having a surface formed with a plurality of binary patterns constituting a multithreaded application program executable by a desktop computer having a central microprocessor, a memory, means for loading said application program into a defined address space of said memory, and a clock-driven periodically-activated interrupt operation, said multithreaded program comprising

a plurality of sets of instructions with each set executable by said microprocessor,

a first of said sets of instructions executable to provide a first thread of execution having control of the central microprocessor,

said first thread of execution being periodically preempted in response to activations of an interrupt operation at predetermined fixed time intervals, and

a second of said sets of instructions executable to provide a second thread of execution to acquire control of the central microprocessor,

each of said threads having direct access to said program memory address space so as to provide fast efficient preemption of one thread by

another thread and switching of control of the central microprocessor back and forth among the threads at a rate so rapid that the threads execute effectively simultaneously.

Analysis:

Claim 20 is a new claim, which is broader in scope than the original claims. There are four elements missing from the claims (the editor, compiler, interrupt means, and return means). These missing elements are described by applicant as being an essential or critical feature of the claimed invention as a whole as evidenced by applicant's repeated reliance on the presence of these elements throughout the originally filed disclosure. Multiple sections within the application make clear that these four elements served integral functions in the overall invention.

Conclusion:

Reject claim 20 as lacking an adequate written description because four elements described as essential or critical are omitted. The omitted elements are: editor, compiler, interrupt means, and return means.

Example 4 : Original claim

Fact Pattern:

The invention is directed to a form of autopilot, described as a "heading lock," which enables a person to maintain directional control over a watercraft without constant manipulation of trolling motor controls. The preferred embodiment, as set forth in the written description and clearly depicted in detailed drawings, employs a compass mounted to the head of the "heading lock" unit, which monitors the direction of the thrust motor. The heading lock is coupled to the trolling motor; in a preferred embodiment, the heading lock is mechanically coupled to the trolling motor. The disclosure specifically notes that the direction of the thrust motor is considered to be the same as the direction of the boat since the trolling motor is mounted on the bow of the boat. The specification indicates that the electronic steering system continues to monitor the current heading of the thrust and also indicates that the heading detector continuously monitors the current heading of the boat. The term "heading" is used interchangeably throughout the written description to refer to both the direction of the trolling motor and the direction of the boat.

Claim:

1. A heading lock coupled to a trolling motor producing a thrust disposed to pull a watercraft, said heading lock comprising:

a steering motor coupled to said trolling motor, said steering motor being disposed to affect the orientation of said trolling motor in response to input signals;

a steering circuit electrically coupled to said steering motor, said steering circuit being disposed to generate said input signals to said steering motor in response to heading signals; and

a heading detector electrically coupled to said steering circuit, said heading detector being disposed to transmit said heading signals to said steering circuit.

Analysis:

Applicant has identified a heading lock comprising a steering system coupled to a trolling motor and a heading detector, as features essential to the operation of the claimed invention. Although the heading lock is preferably mechanically coupled to the trolling motor, the applicant does not describe the type of coupling as essential to the claimed invention as a whole. A search of the prior art shows that various means for coupling a heading lock to a trolling motor are conventional in the art. The claim is drawn to a single embodiment. Although there is no reduction to practice of the claimed invention, the claimed invention is clearly depicted in detailed drawings.

Conclusion:

The claim is adequately described.

Example 5: Flow Diagrams

Fact Pattern:

The specification is directed to a mechanism for controlling the mode of operation of a modem. A modem is used for modulating and demodulating signals, both analog and digital, over telephone lines. It has two modes: (1) a transparent mode, in which the modem performs the modulation-demodulation function, and (2) a command mode, in which the modem responds to predetermined commands and performs operations by executing a set of instructions stored in Read-Only-Memory (ROM) or firmware. An escape command tells the modem when to switch between transparent and command modes.

The application claims an improved mechanism for detecting an escape command by a modem. The decision making capability and timing means preferably reside in a microprocessor, preferably a Z-8 type microprocessor. The specification discloses logic flow diagrams and provides a detailed functional recitation that describes how to program computers to detect an escape command, but the specification does not provide a computer program listing with source code. The specification describes the escape sequence as one full second of no data, followed by the predetermined escape command, followed by another full second of no data.

Claim:

1. In a modem including a data input port for connecting said modem to a utilization device, and a telephone port for connecting said modem to a

telephone line, said modem being of the type having two distinct modes of operation:

(a) a transparent mode of operation for which said modem provides modulated signals to said telephone port in response to data signals provided to said data input port; and

(b) a command mode of operation for which said modem responds to said data signals provided to said data input port as instructions to said modem;

said modem including means defining a predetermined sequence of said data signals as an escape character; the improvement comprising:

timing means for detecting each occurrence of a passage of a predetermined period of time after provision of one of said data signals to said data input port; and

means, operative when said modem is in said transparent mode of operation, for detecting provision of said predetermined sequence of said data signals, and for causing said modem to switch to said command mode of operation, if and only if said predetermined sequence of data signals occurs contiguous in time with at least one said occurrence of said passage of said predetermined period of time during which none of said data signals are provided to said data input port.

Analysis:

After a review of the full content of the specification, the examiner finds that a modem having two modes of operation (transparent and

command), a timing means, and a means for detecting an escape sequence and causing the modem to switch from the transparent to the command mode are essential to the operation and function of the claimed invention. The specification does not describe a particular timing means or means for detecting the escape command and switching to the command mode. The claim is drawn to a genus. A search of the prior art indicates that the structure of the hardware required is conventional, and that one skilled in the art would know how to program a microprocessor to perform the necessary steps described in the specification. A review of the art indicates that there is no substantial variation among the species within the genus. Although no embodiments have been actually reduced to practice, a review of the specification shows that the claimed invention has been reduced to drawings in view of the detailed functional flow diagrams. Since the claimed invention is supported by conventional hardware structure and because there is a functional description of what the software does to operate the computer, there is sufficient description of the claimed invention. Disclosing a microprocessor capable of performing certain functions is sufficient to satisfy the requirement of section 112, first paragraph, when one skilled in the relevant art would understand what is intended and know how to carry it out.

Conclusion:

The claimed invention has been adequately described.

Biotechnology Examples

Example 6: Genes

Specification: The specification describes an isolated cDNA fragment (SEQ ID NO: 1; a 100mer) obtained from a human glioblastoma cDNA library. SEQ ID NO: 1 is asserted to be homologous to a known DNA molecule that encodes the extracellular domain of a glial specific G-coupled protein receptor whose function is associated with glial cell differentiation. The observed homology is sufficient to support a conclusion that SEQ ID NO: 1 would be glial specific. Further, it would be reasonable to infer that a G-coupled protein receptor encoded by a cDNA that comprised SEQ ID NO: 1 would be involved in the regulation of glial cell differentiation. In the description, applicant defines a "gene" as including naturally occurring regulatory elements and untranslated regions necessary and sufficient to mediate the expression of a cDNA comprising SEQ ID NO: 1. The specification describes methods for cloning nucleic acids that encode full-length glial specific G coupled protein receptors. The specification also discloses that SEQ ID NO: 1 can be used as a probe for identifying the presence of nucleic acids encoding glial specific G-coupled protein receptors in mammals. Glial specific G-coupled protein receptors are disclosed as useful in drug discovery methods to identify agents that regulate glial differentiation. The specification defines a probe as consisting of SEQ ID NO: 1 and between five to 10 additional nucleotides on either end of SEQ ID NO: 1.

Claim:

An isolated gene comprising SEQ ID NO: 1.

Analysis:

A review of the specification indicates that elements which are not particularly described, including regulatory elements and untranslated regions, are essential to the function of the claimed invention because applicant's definition of "gene" requires them. Additionally, SEQ ID NO: 1 is disclosed as being essential to the function of the claimed invention. The art indicates that the structure of genes with naturally occurring regulatory elements and untranslated regions is empirically determined. For example, the structural elements of "gene" mediating the expression of a particular protein in the liver may be different than the structural elements of the "gene" mediating the expression of the same protein in the brain. Therefore the structure of these elements which applicant considers as being essential to the function of the claim are not conventional in the art.

The claim is drawn to a genus, i.e., any gene which comprises SEQ ID NO: 1.

A search of the prior art indicates that SEQ ID NO: 1 is otherwise novel and unobvious, and no associated genomic clones have been identified.

There is no actual reduction to practice of the claimed invention, clear depiction of the claimed invention in the drawings or complete detailed description of the structure.

Considering all disclosed distinguishing identifying characteristics, there is a disclosure of partial structure (SEQ ID NO: 1) as well as the function of the gene as coding for a G-coupled protein receptor.

However, there is no known or disclosed correlation between this function and the structure of the non-described regulatory elements and untranslated regions of the gene. Furthermore, there is no additional disclosure of physical and/or chemical properties. Weighing all factors in view of the level of knowledge and skill in the art, one skilled in the art would not recognize from the disclosure that the applicant was in possession of the genus of genes which comprise SEQ ID NO: 1.

Conclusion:

Reject claim 1 under 35 USC 112 first paragraph as lacking an adequate written description. The examiner should make a rejection following a similar type of reasoning as that set forth above.

Note: Applicant may overcome this rejection by claiming a probe which consists essentially of SEQ ID NO: 1, since the specification teaches that a probe can have no more than 10 additional nucleic acid residues at either end of the molecule. The examiner should make an express determination that "consisting essentially of" admits of no more than 10 additional residues at either end of the molecule.

Example 7: EST

Specification: The specification discloses SEQ ID NO: 16 which is a partial cDNA. The specification does not address whether the cDNA crosses an exon/intron splice junction. The specification discloses that this sequence will specifically hybridize with the complement of the coding sequence of a gene of an infectious yeast. The presence of the nucleic acid detected by hybridization with the complement of the coding sequence is useful for identifying yeast infections. Example 1 of the specification describes an experiment where SEQ ID NO: 16 was determined following characterization of a cDNA clone isolated from a cDNA library.

Claim:

An isolated DNA comprising SEQ ID NO: 16.

Analysis:

A review of the full content of the specification indicates SEQ ID NO: 16 is essential to the operation and function of the claimed invention. The specification indicates that the presence of DNA that hybridizes with SEQ ID NO: 16 is indicative of a yeast infection.

A review of the language of the claim indicates that the claim is drawn to a genus, i.e., any nucleic acid that minimally contains SEQ ID NO: 16 within it including any full length gene which contains the sequence, any fusion constructs or cDNAs.

The search indicates that SEQ ID NO: 16 is a novel and unobvious sequence.

There is a single species explicitly disclosed (a molecule consisting of SEQ ID NO: 16 that is within the scope of the claimed genus).

There is actual reduction to practice of the disclosed species.

The disclosure of a single disclosed species may provide an adequate written description of a genus when the species disclosed is representative of the genus. The present claim encompasses full-length genes and cDNAs that are not further described. There is substantial variability among the species of DNAs encompassed within the scope of the claims because SEQ ID NO: 16 is only a fragment of any full-length gene or cDNA species. When reviewing a claim that encompasses a widely varying genus, the examiner must evaluate any necessary common attributes or features. In the case of a partial cDNA sequence that is claimed with open language (comprising), the genus of, e.g., "A cDNA comprising [a partial sequence]," encompasses a variety of subgenera with widely varying attributes. For example, a cDNA's principle attribute would include its coding region. A partial cDNA that did not include a disclosure of any open reading frame (ORF) of which it would be a part, would not be representative of the genus of cDNAs because no information regarding the coding capacity of any cDNA molecule would be disclosed. Further, defining "the" cDNA in functional terms would not suffice in the absence of a disclosure of structural features or elements of a cDNA that would encode a protein having a stated function.

A description of a genus of cDNAs may be achieved by means of a recitation of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a

substantial portion of the genus. Regents of the University of California v. Eli Lilly & Co., 119 F3d 1559, 1569, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997).

Here, the specification discloses only a single common structural feature shared by members of the claimed genus, i.e., SEQ ID NO: 16. Since the claimed genus encompasses genes yet to be discovered, DNA constructs that encode fusion proteins, etc., the disclosed structural feature does not "constitute a substantial portion" of the claimed genus. Therefore, the disclosure of SEQ ID NO: 16 does not provide an adequate description of the claimed genus.

Weighing all factors, 1) partial structure of the DNAs that comprise SEQ ID NO: 16, 2) the breadth of the claim as reading on genes yet to be discovered in addition to numerous fusion constructs and cDNAs, 3) the lack of correlation between the structure and the function of the genes and/or fusion constructs; in view of the level of knowledge and skill in the art, one skilled in the art would not recognize from the disclosure that the applicant was in possession of the genus of DNAs which comprise SEQ ID NO: 16.

Conclusion: The written description requirement is not satisfied.

Caveat: *In situations where the specification indicates that the SEQ ID NO: is a full-length cDNA open reading frame and the claim* ***cannot*** *read on a gene, the claimed invention would meet the written description requirement.*

Example 8: <u>DNA fragment Encoding a Full Open Reading Frame (ORF)</u>

Specification: The specification discloses that a cDNA library was prepared from human kidney epithelial cells and 5000 members of this library were sequenced and open reading frames were identified. The specification discloses a Table that indicates that one member of the library having SEQ ID NO: 2 has a high level of homology to a DNA ligase. The specification teaches that this complete ORF (SEQ ID NO: 2) encodes SEQ ID NO: 3. An alignment of SEQ ID NO: 3 with known amino acid sequences of DNA ligases indicates that there is a high level of sequence conservation between the various known ligases. The overall level of sequence similarity between SEQ ID NO: 3 and the consensus sequence of the known DNA ligases that are presented in the specification reveals a similarity score of 95%. A search of the prior art confirms that SEQ ID NO: 2 has high homology to DNA ligase encoding nucleic acids and that the next highest level of homology is to alpha-actin. However, the latter homology is only 50%. Based on the sequence homologies, the specification asserts that SEQ ID NO: 2 encodes a ligase.

Claim 1: An isolated and purified nucleic acid comprising SEQ ID NO: 2.

Analysis:

A review of the full content of the specification indicates SEQ ID NO: 2 is essential to the operation and function of the claimed invention. The specification indicates that SEQ ID NO: 2 encodes a protein that would be expected to act as a DNA ligase.

A review of the language of the claim indicates that the claim is drawn to a genus, i.e., any nucleic acid that minimally contains SEQ ID NO: 2. The claim is drawn to a nucleic acid comprising a full open reading frame. The claimed nucleic acid does not read on a genomic sequence because full-length mammalian cDNAs would not be expected to contain introns or transcriptional regulatory elements such as promoters that are found in genomic DNA. The claim reads on the claimed ORF in any construct or with additional nucleic acid residues placed at either end of the ORF.

The search indicates that SEQ ID NO: 2 is a novel and unobvious sequence.

There is a single species explicitly disclosed (a molecule consisting of SEQ ID NO: 2 that is within the scope of the claimed genus).

There is actual reduction to practice of the disclosed species.

One of skill in the art can readily envisage nucleic acid sequences which include SEQ ID NO: 2 because e.g. SEQ ID NO: 2 can be readily embedded in known vectors. Although there may be substantial variability among the species of DNAs encompassed within the scope of the claim because SEQ ID NO: 2 may be combined with sequences known in the art,

e.g. expression vectors, the necessary common attribute is the ORF (SEQ ID NO: 2).

Weighing all factors including (1) that the full length ORF (SEQ ID NO: 2) is disclosed and (2) that any substantial variability within the genus arises due to addition of elements that are not part of the inventor's particular contribution, taken in view of the level of knowledge and skill in the art, one skilled in the art would recognize from the disclosure that the applicant was in possession of the genus of DNAs that comprise SEQ ID NO: 2.

Conclusion: The written description requirement is satisfied.

Example 9: Hybridization

Specification: The specification discloses a single cDNA (SEQ ID NO:1) which encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity. The specification includes an example wherein the complement of SEQ ID NO: 1 was used under highly stringent hybridization conditions (6XSSC and 65 degrees Celsius) for the isolation of nucleic acids that encode proteins that bind to dopamine receptor and stimulate adenylate cyclase activity. The hybridizing nucleic acids were not sequenced. They were expressed and several were shown to encode proteins that bind to a dopamine receptor and stimulate adenylate cyclase activity. These sequences may or may not be the same as SEQ ID NO: 1.

Claim:

An isolated nucleic acid that specifically hybridizes under highly stringent conditions to the complement of the sequence set forth in SEQ ID NO: 1,

wherein said nucleic acid encodes a protein that binds to a dopamine receptor and stimulates adenylate cyclase activity.

Analysis:

A review of the full content of the specification indicates that the essential feature of the claimed invention is the isolated nucleic acid that hybridizes to SEQ ID NO: 1 under highly stringent conditions and encodes a protein with a specific function. The art indicates that hybridization techniques using a known DNA as a probe under highly stringent conditions were conventional in the art at the time of filing.

The claim is drawn to a genus of nucleic acids all of which must hybridize with SEQ ID NO: 1 and must encode a protein with a specific activity.

The search of the prior art indicates that SEQ ID NO: 1 is novel and unobvious.

There is a single species disclosed (a molecule consisting of SEQ ID NO: 1) that is within the scope of the claimed genus.

There is actual reduction to practice of the disclosed species.

Now turning to the genus analysis, a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because the highly stringent hybridization conditions set forth in the claim yield structurally similar DNAs. Thus, a representative number of species is disclosed, since highly stringent hybridization conditions in combination with the coding function of DNA and the level of

skill and knowledge in the art are adequate to determine that applicant was in possession of the claimed invention.

Conclusion: The claimed invention is adequately described.

Example 10: Process claim

Specification: The specification teaches that SEQ ID NO: 10 is an EST. The specification also teaches that SEQ ID NO: 10 is a chromosome marker and that any DNA which hybridizes under specified stringent conditions to SEQ ID NO: 10 will be useful as a marker for detecting the presence of Burkitt's lymphoma. The specification also teaches how to produce DNAs including genomic DNAs which hybridize to SEQ ID NO: 10 and isolation of said DNAs. The specification presents an example where a genomic DNA is probed with SEQ ID NO: 10 under the specified stringent conditions (6XSSC and 65 degrees Celsius) and the genomic DNA which hybridizes under these conditions is isolated and is sequenced. The sequence of this genomic clone is represented by SEQ ID NO: 11.

Claim:

Claim 1: A process for producing an isolated polynucleotide comprising hybridizing SEQ ID NO: 10 to genomic DNA in 6XSSC and 65° C and isolating the DNA polynucleotide detected with SEQ ID NO: 10.

Claim 2: An isolated DNA that hybridizes with SEQ ID NO: 10.

Analysis:

Claim 1:

A review of the full content of the specification indicates that the essential feature of the claimed invention is a process of obtaining a nucleic acid sequence which is identified by a probe that hybridizes to SEQ ID NO:10 and a polynucleotide that hybridizes with SEQ ID NO: 10. The

specification and the general state of the art indicate that the general process of producing nucleic acids through hybridization with probes was routine at the time of filing.

The claim is drawn to a genus i.e., a process of hybridizing to genomic DNA with SEQ ID NO: 10 and isolating the DNA which hybridizes under specific conditions to said sequence.

The search indicates that SEQ ID NO: 10 and SEQ ID NO: 11 are novel and unobvious sequences. Therefore, under the examination guidelines of *In re Ochiai* and *In re Brouwer*, the method of making a novel and unobvious product is also novel and unobvious.

The specification presents an example where a single species has been reduced to practice, i.e., isolation of SEQ ID NO: 11 based on hybridization with SEQ ID NO: 10. Therefore the disclosed species within the genus has been adequately described. Now turning to the genus analysis, the art indicates that there is no substantial variation within the genus because of the stringency of hybridization conditions which yields structurally similar molecules. The single disclosed species is representative of the genus because reduction to practice of this species, considered along with the defined hybridization conditions and the level of skill and knowledge in the art, are sufficient to allow the skilled artisan to recognize that applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus.

Claim 2:

The claim is drawn to a genus of nucleic acids, all of which must hybridize to SEQ ID NO: 10. The claim does not specify any stringency conditions. The claim is broad and reads on virtually any nucleic acid.

There is a species disclosed, SEQ ID NO: 11. The art indicates that there is substantial variation within the genus because the lack of stringency of hybridization conditions would be expected to yield structurally unrelated nucleic acid molecules. The single disclosed species is not representative of the genus because there is no structural attribute or feature that is common to the members of the genus.

Conclusion:

Claim 1 is adequately described.

Claim 2 should be rejected as lacking adequate written description following the analysis described above.

Note: Applicant may overcome the written description rejection of the product by, for example, substituting claim 2 with a product by process claim such as the one below.

Claim 2. *The isolated DNA polynucleotide prepared according to the process of claim 1.*

Example 11: Allelic Variants

Specification: The specification discloses a DNA, SEQ ID NO: 1, said to encode a cell surface receptor for adenovirus. The cell surface receptor is designated protein X and its sequence is given as SEQ ID NO:2. The specification states that the invention includes alleles of the DNA that include single nucleotide polymorphisms (SNPs). No allelic sequence information is disclosed, but the specification states that allelic variants of SEQ ID NO: 1 can be obtained, e.g., by hybridizing SEQ ID NO: 1 to a DNA library made from the species of organism that yielded SEQ ID NO: 1.

Claims:

1. An isolated DNA that encodes protein X (SEQ ID NO: 2).

2. An isolated allele of the DNA according to claim 1, which allele encodes protein X (SEQ ID NO: 2).

3. An isolated allele of SEQ ID NO: 1.

Analysis:

Claim 1:

Claim 1 is drawn to the genus of DNAs that encode amino acid sequence SEQ ID NO:2, i.e., all sequences degenerately related by a genetic code table to SEQ ID NO:1. Although only one specie within the genus is disclosed, SEQ ID NO:1, a person of skill in the art could readily envision all the DNAs degenerate to SEQ ID NO:1 by using a genetic code table. One of skill in the art would conclude that applicant was in possession of the

genus based on the specification and the general knowledge in the art concerning a genetic coding table.

Claim 2:

Claim 2 is drawn to a subgenus of allelic DNAs that encode amino acid sequence SEQ ID NO: 2. The specification does not provide any particular definition for the term allele. In this circumstance, the meaning of the term is the ordinary usage in the art. The ordinary meaning of the term allele is one of two or more alternate forms of a gene occupying the same locus in a particular chromosome or linkage structure and differing from other alleles of the locus at one or more mutational sites. See, Rieger et al., *Glossary of Genetics* (1991), p. 16. The alleles in claim 2 are "strictly neutral" because they encode identical proteins, and make no difference to phenotype. See, Rieger et al., p. 17. Although the standard definition refers to genomic sequences and the claims are directed to DNAs, a reasonable interpretation is that the claim is directed to DNAs that include naturally occurring mutational site(s).

The specification discloses only one allele within the scope of the genus: SEQ ID NO:1. The specification proposes to discover other members of the genus by using a hybridization procedure. There is no description of the mutational sites that exist in nature, and there is no description of how the structure of SEQ ID NO: 1 relates to the structure of any strictly neutral alleles. The general knowledge in the art concerning alleles does not provide any indication of how the structure of one allele is representative of unknown alleles. The nature of alleles is that they are variant structures, and in the present state of the art the structure of one does

not provide guidance to the structure of others. The common attributes of the genus are not described. One of skill in the art would conclude that applicant was not in possession of the claimed genus because a description of only one member of this genus is not representative of the variants of the genus and is insufficient to support the claim.

Claim 3:

Claim 3 is drawn to the genus including all DNA alleles of SEQ ID NO: 1. The specification does not provide any particular definition for the term allele. In this circumstance, the meaning of the term is the ordinary usage in the art. The ordinary meaning of the term allele is one of two or more alternate forms of a gene occupying the same locus in a particular chromosome or linkage structure and differing from other alleles of the locus at one or more mutational sites. See, Rieger et al., *Glossary of Genetics* (1991), p. 16. The Rieger reference discloses that there are at least seven different kinds of allele in addition to the "strictly neutral" type discussed above for Claim 2. See, Rieger, pp. 16-17 (amorphs, hypomorphs, hypermorphs, antimorphs, neomorphs, isoalleles, and unstable alleles). The alleles are distinguished by the effect their different structures have on phenotype. According to Rieger, alleles may differ functionally according to their distinct structures. For example, they may differ in the amount of biological activity the protein product may have, may differ in the amount of protein produced, and may even differ in the kind of activity the protein product will have.

The specification discloses only one allele within the scope of the genus: SEQ ID NO:1. The specification proposes to discover other

members of the genus by using a hybridization procedure. There is no description of the mutational sites that exist in nature, and there is no description of how the structure of SEQ ID NO: 1 relates to the structure of different alleles. In addition, according to the standard definition, the genus includes members that would be expected to have widely divergent functional properties. The general knowledge in the art concerning alleles does not provide any indication of how the structure of one allele is representative of other unknown alleles having concordant or discordant functions. The common attributes of the genus are not described and the identifying attributes of individual alleles, other than SEQ ID NO:1, are not described. The nature of alleles is that they are variant structures where the structure and function of one does not provide guidance to the structure and function of others. According to these facts, one of skill in the art would conclude that applicant was not in possession of the claimed genus because a description of only one member of this genus is not representative of the variants of the genus and is insufficient to support the claim.

Conclusions:

Claim 1:

Claim 1 should not be rejected under the written description requirement.

Claim 2:

Claim 2 should be rejected under the written description requirement. An analysis similar to the one set forth above could be used. Since the Office has the burden of presenting evidence to support its position, see

MPEP 2163.04, a reference should be relied on as authority for the Office's interpretation of the claim term "allele."

Claim 3:

Claim 3 should be rejected under the written description requirement. An analysis similar to the one set forth above could be used. Since the Office has the burden of presenting evidence to support its position, see MPEP 2163.04, a reference should be relied on as authority for the Office's interpretation of the claim term "allele."

For the rejections of claims 2 and 3, the Office interpretation of "allele" should be supported by a reference, rather than by taking "notice," because the interpretation is the principle evidence supporting the rejection. See MPEP 2144.03 (For further views on official notice, see *In re Ahlert*, 424 F.2d 1088, 1091 165 USPQ 418, 420 - 421 (CCPA 1970) ("[A]ssertions of technical facts in areas of esoteric technology must always be supported by citation of some reference work" and "allegations concerning specific `knowledge' of the prior art, which might be peculiar to a particular art should also be supported." Furthermore the applicant must be given the opportunity to challenge the correctness of such assertions and allegations. "The facts so noticed serve to `fill the gaps' which might exist in the evidentiary showing" and should not comprise the principle evidence upon which a rejection is based.); see also, *In re Barr*, 444 F.2d 588, 170 USPQ 330 (CCPA 1971) (scientific journal references were not used as a basis for taking judicial notice that controverted phrases were art - recognized because the court was not sure that the meaning of the term at issue was indisputable among reasonable men); *In re Eynde*, 480 F.2d 470, 178 USPQ

470,474 (CCPA 1973) ("The facts constituting the state of the art are normally subject to the possibility of rational disagreement among reasonable men and are not amenable to the taking of [judicial] notice.").)

Example 12: Bioinformatics

Specification: The specification discloses a process for identifying and selecting biological compounds that are present in a biological system in a tissue specific manner. In the disclosed process the expression level of a set of compounds is quantitatively determined in multiple tissues within an organism. The expression level data is then graphically displayed in such a manner that compounds that are differentially expressed are easily identified. An artisan interested in identifying a compound that is expressed at a high level in one tissue and at a different level in a second tissue may easily select compounds that are expressed in a tissue specific manner based on the displayed information. The specification indicates that the compounds to be detected encompass DNA, RNA and proteins as well as metabolites. The specification does not provide any particular examples, but discloses that the expression levels can be determined by any analytical method consistent with the class of compounds being detected. This type of measurement requires actual physical steps.

Claim:

A computer-implemented method of selecting tissue specific compounds, said method comprising the steps of:

(a) analyzing the expression level of compounds in a first and second tissue and obtaining expression level data for each of said compounds;

(b) inputting the expression level data obtained in step a) into a computer;

(c) displaying a first axis corresponding to the expression level of each of said compounds in said first tissue;

(d) displaying a second axis substantially perpendicular to said first axis, said second axis corresponding to the expression level data of each of said compound in said second sample

(e) displaying a mark at a position, wherein said position is selected relative to said first axis in accordance with an expression level of each of said compound in said first sample and relative to said second axis in accordance with the expression of said compound in said second sample; and

(f) selecting a compound of interest based on the position of the mark.

Analysis:

A review of the full content of the specification indicates that obtaining, inputting, and displaying the expression level of compounds is essential to the operation of the claimed invention.

A search of the prior art indicates that obtaining the expression level data of compounds is conventional in the art, and that data display devices and associated support algorithms are well known in the art.

A review of the claim indicates that the claim is drawn to a generic environment for the display of compounds in a tissue specific manner.

Since there is no species claimed or disclosed, the claim is analyzed as a claim drawn to a single embodiment. There is no actual reduction to practice of the claimed invention, or clear depiction of the claimed invention

in detailed drawings. However, reading the specification in light of the knowledge and level of skill in the art, the specification discloses the complete steps of the claimed process. See In re Hayes Microcomputer Products Inc. Patent Litigation, 982 F2d. 1527, 1534-35, 25 USPQ2d 1241, 1246 (Fed. Cir. 1992), where the court stated,

> One skilled in the art would know how to program a microprocessor to perform the necessary steps desired in the specification. Thus, an inventor is not required to describe every detail of his invention. An applicant's disclosure obligation varies according to the art to which the invention pertains.

In this fact situation, the art is sufficiently developed so as to put one of skill in the art in possession of the complete steps of the process. In other words, one skilled in the relevant art would understand what is intended by the claimed invention and know how to carry it out.

Conclusion: There is adequate written description for what is claimed.

Example 13: Protein Variant

Specification: The specification describes a protein isolated from liver. A working example shows that the isolated protein was sequenced and determined to consist of SEQ ID NO: 3. The isolated protein was additionally characterized as being 65 kD in molecular weight and having tumor necrosis activity. The specification states that the invention provides variants of SEQ ID NO: 3 having one or more amino acid substitutions, deletions, insertions and/or additions. No further description of the variants is provided. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and/or additions are routine in the art. The specification does not define when a protein ceases to be a variant of SEQ ID NO: 3.

Claims:

1. An isolated protein having SEQ ID NO: 3.

2. An isolated variant of the protein of claim 1.

Analysis:

Claim 1:

A search of the prior art indicates that SEQ ID NO: 3 is novel and nonobvious. The claim is directed to a genus of proteins that comprise SEQ ID NO: 3. One member of the genus, SEQ ID NO: 3, is described by a complete structure.

There is relatively little variation among the species within the genus because each member of the genus shares SEQ ID NO: 3 as a necessary common feature. The single disclosed example is representative of the claimed genus because taken in view of the general knowledge in the art, the disclosure is sufficient to show that one of skill in the art would conclude that applicant was in possession of the claimed genus.

Claim 2:

This is a genus claim. According to the specification, the term variant means a protein having one or more amino acid substitutions, deletions, insertions and/or additions made to SEQ ID NO: 3. The specification and claim do not indicate what distinguishing attributes shared by the members of the genus. The specification and claim do not place any limit on the number of amino acid substitutions, deletions, insertions and/or additions that may be made to SEQ ID NO: 3. Thus, the scope of the claim includes numerous structural variants, and the genus is highly variant because a significant number of structural differences between genus members is permitted. Although the specification states that these types of changes are routinely done in the art, the specification and claim do not provide any guidance as to what changes should be made. Structural features that could distinguish compounds in the genus from others in the protein class are missing from the disclosure. No common structural attributes identify the members of the genus. The general knowledge and level of skill in the art do not supplement the omitted description because specific, not general, guidance is what is needed. Since the disclosure fails to describe the common attributes or characteristics that identify members of the genus, and because the genus is highly variant, SEQ ID NO: 3 alone is insufficient to

describe the genus. One of skill in the art would reasonably conclude that the disclosure fails to provide a representative number of species to describe the genus. Thus, applicant was not in possession of the claimed genus.

Conclusions:

Claim 1:

The claimed subject matter is adequately described. A rejection under the written description requirement should not be entered.

Claim 2:

The claimed subject matter is not supported by an adequate written description because a representative number of species have not been described. A rejection under the written description requirement, relying on the analysis set out above, should be entered.

Example 14: Product by Function

Specification: The specification exemplifies a protein isolated from liver that catalyzes the reaction of A $\longrightarrow$ B. The isolated protein was sequenced and was determined to have the sequence as set forth in SEQ ID NO: 3. The specification also contemplates but does not exemplify variants of the protein wherein the variant can have any or all of the following: substitutions, deletions, insertions and additions. The specification indicates that procedures for making proteins with substitutions, deletions, insertions and additions is routine in the art and provides an assay for detecting the catalytic activity of the protein.

Claim:

A protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO: 3 and catalyze the reaction of A $\longrightarrow$ B.

Analysis:

A review of the full content of the specification indicates that a protein having SEQ ID NO: 3 or variants having 95% identity to SEQ ID NO: 3 and having catalytic activity are essential to the operation of the claimed invention. The procedures for making variants of SEQ ID NO: 3 are conventional in the art and an assay is described which will identify other proteins having the claimed catalytic activity. Moreover, procedures for making variants of SEQ ID NO: 3 which have 95% identity to SEQ ID NO: 3 and retain its activity are conventional in the art.

A review of the claim indicates that variants of SEQ ID NO: 3 include but are not limited to those variants of SEQ ID NO: 3 with substitutions, deletions, insertions and additions; but all variants must possess the specified catalytic activity and must have at least 95% identity to the SEQ ID NO: 3. Additionally, the claim is drawn to a protein which **comprises** SEQ ID NO: 3 or a variant thereof that has 95% identity to SEQ ID NO: 3. In other words, the protein claimed may be larger than SEQ ID NO: 3 or its variant with 95% identity to SEQ ID NO: 3. It should be noted that "having" is open language, equivalent to "comprising".

The claim has two different generic embodiments, the first being a protein which comprises SEQ ID NO: 3 and the second being variants of SEQ ID NO: 3. There is a single species disclosed, that species being SEQ ID NO: 3.

A search of the prior art indicates that SEQ ID NO: 3 is novel and unobvious.

There is actual reduction to practice of the single disclosed species. The specification indicates that the genus of proteins that must be variants of SEQ ID NO: 3 does not have substantial variation since all of the variants must possess the specified catalytic activity and must have at least 95% identity to the reference sequence, SEQ ID NO: 3. The single species disclosed is representative of the genus because all members have at least 95% structural identity with the reference compound and because of the presence of an assay which applicant provided for identifying all of the at least 95% identical variants of SEQ ID NO: 3 which are capable of the specified catalytic activity. One of skill in the art would conclude that

applicant was in possession of the necessary common attributes possessed by the members of the genus.

Conclusion: The disclosure meets the requirements of 35 USC §112 first paragraph as providing adequate written description for the claimed invention.

Example 15: Antisense

Specification: The specification discloses a messenger RNA sequence, SEQ ID NO: 1, which encodes human growth hormone. The specification states that the invention includes antisense molecules that inhibit the production of human growth hormone. The specification describes an art-recognized method of screening for antisense molecules that is called "gene walking." Gene walking is said to involve obtaining antisense oligonucleotides that are complementary to the target sequence.

Claim:

An antisense oligonucleotide complementary to a messenger RNA having SEQ ID NO: 1 and encoding human growth hormone, wherein said oligonucleotide inhibits the production of human growth hormone.

Analysis:

A review of the full content of the specification indicates that the complement of SEQ ID NO: 1 is essential to the operation of the claimed invention. The general knowledge in the art is that any full-length complement of a target mRNA inhibits the function of the mRNA and is therefore an antisense oligonucleotide. Thus, one of skill in the art would view applicant's disclosure of a coding sequence, with the statement that the invention includes antisense oligonucleotides, as an implicit disclosure that the full-length complement of SEQ ID NO: 1 is an antisense oligonucleotide.

It is generally accepted in the art that oligonucleotides complementary to a messenger RNA, including fragments of the full-length complement, have antisense activity when they match accessible regions on the target mRNA. Generally, the closer the complementary fragment is to full length, the greater the likelihood it will have antisense activity. In addition, oligos that retain complementarity to the Shine-Delgarno sequence usually have antisense activity.

The claim is drawn to the genus of antisense molecules that inhibit the production of human growth hormone encoded by SEQ ID NO: 1. There is a single species described with a complete structure, i.e., the full-length complement of SEQ ID NO: 1. In addition to the full-length complement, the genus includes fragments of the complement that retain antisense activity.

The procedures for making oligonucleotide fragments of the SEQ ID NO: 1 complement are conventional, e.g., any specified fragment can be ordered from a commercial synthesizing service. The procedures for screening for antisense activity are also conventional, and the specification describes the assay needed to do gene walking. The experience accumulated in the art with gene walking is that numerous regions of a target are accessible, that these regions are identified routinely, and that antisense oligonucleotides are complementary to these accessible regions. The full-length complement and longer fragments match multiple accessible regions; shorter fragments match fewer accessible regions.

When considering the distinguishing characteristics of the claimed invention, the sequence provided in the specification defines and limits the

structure of any effective antisense molecules. The specification also teaches the functional characteristics of the claimed invention as well as a routine art recognized method of making and screening for the claimed invention. Considering the specification's disclosure of:

(1) the sequence (SEQ ID NO: 1) which defines and limits the structure of any effective antisense molecules such that one skilled in the art would be able to immediately envisage members of the genus embraced by the claim, and

(2) the functional characteristics of the claimed invention as well as a routine art-recognized method of screening for antisense molecules which provide further distinguishing characteristics of the claimed invention, along with

(3) the general level of knowledge and skill in the art,

one skilled in the art would conclude that applicant was in possession of the invention.

Conclusion: The claimed invention is adequately described.

Example 16: Antibodies

Specification: The specification teaches that antigen X has been isolated and is useful for detection of HIV infections. The specification teaches antigen X as purified by gel filtration and provides characterization of the antigen as having a molecular weight of 55 KD. The specification also provides a clear protocol by which antigen X was isolated. The specification contemplates but does not teach in an example antibodies which specifically bind to antigen X and asserts that these antibodies can be used in immunoassays to detect HIV. The general knowledge in the art is such that antibodies are structurally well characterized. It is well known that all mammals produce antibodies and they exist in five isotypes, IgM, IgG, IgD, IgA and IgE. Antibodies contain an effector portion which is the constant region and a variable region that contains the antigen binding sites in the form of complementarity determining regions and the framework regions. The sequences of constant regions as well as the variable regions subgroups (framework regions) from a variety of species are known and published in the art. It is also well known that antibodies can be made against virtually any protein.

Claim: An isolated antibody capable of binding to antigen X.

Analysis:

A review of the full content of the specification indicates that antibodies which bind to antigen X are essential to the operation of the claimed invention. The level of skill and knowledge in the art of antibodies at the time of filing was such that production of antibodies against a well-

characterized antigen was conventional. This is a mature technology where the level of skill is high and advanced.

The claim is directed to any antibody which is capable of binding to antigen X.

A search of the prior art indicates that antigen X is novel and unobvious.

Considering the routine art-recognized method of making antibodies to fully characterized antigens, the well defined structural characteristics for the five classes of antibody, the functional characteristics of antibody binding, and the fact that the antibody technology is well developed and mature, one of skill in the art would have recognized that the spectrum of antibodies which bind to antigen X were implicitly disclosed as a result of the isolation of antigen X.

Conclusion: The disclosure meets the requirement under 35 USC 112 first paragraph as providing an adequate written description of the claimed invention.

Example 17: Genus-species with widely varying species

Specification: The specification discloses the rat cDNA sequences for proinsulin and pre-proinsulin and a method for determining the corresponding human and other mammalian insulin cDNA sequences. However, the specification does not disclose any actual cDNA sequence other than the rat proinsulin and pre-proinsulin sequence. The specification discloses that one human proinsulin amino acid (but not cDNA) sequence was known at the time of filing. The art recognized that the sequence of human insulin proteins, and therefore also cDNAs, would probably vary among individuals. The specification also discloses that pre-proinsulin is post translationally modified to form proinsulin, and that proinsulin is cleaved to form insulin.

Claims:

Claim 1. An isolated mammalian cDNA encoding insulin.

Claim 2. The isolated cDNA of claim 1 wherein the mammalian cDNA is human.

Analysis: The examiner should analyze claim 2 first because it is drawn to a subgenus of the genus of claim 1.

Claim 2:

A review of the full content of the specification indicates that human cDNA molecules that encode insulin are essential to the operation/function of the invention.

Claim 2 is directed to a genus of human cDNA which encodes insulin.

There is no species of human insulin cDNA disclosed.

Based upon art published after applicant's filing date there is expected to be variation among the species of cDNA which encode human insulin because the sequence of human insulin proteins, and therefore also human insulin cDNAs, would be expected to vary among individuals.

The specification discloses only the sequence of a single human proinsulin protein, and does not disclose any human cDNA sequence at all.

In addition, there is no evidence on the record of a relationship between the structure of rat insulin cDNA and the structure of insulin cDNAs from humans or other mammals that would provide any reliable information about the structure of other insulin cDNAs on the basis of the rat insulin cDNA.

There is no evidence on the record that the disclosed rat cDNA proinsulin sequence had a known structural relationship to the human cDNA sequence, or to other mammalian cDNA sequences; the specification discloses only a single human proinsulin (protein) sequence; the art indicated that human proinsulin proteins were expected to be variable in structure; and there is expected to be variation among human cDNAs that

encode a given human proinsulin. In view of the these considerations, a person of skill in the art would not have viewed the teachings of the specification as sufficient to show that the applicant was in possession of the claimed human cDNA.

Claim 1:

Claim 1 is directed to a genus of mammalian cDNAs which encode insulin. The specification evidences actual reduction to practice of the rat cDNA sequences for proinsulin and preproinsulin, but does not disclose any other cDNA sequences. The art indicates that there is likely to be substantial variation among the species within the genus of cDNAs that encode mammalian insulins because the sequences of the mammalian insulin proteins, and therefore the mammalian cDNAs, would be expected to vary among species.

The specification discloses a method for determining the corresponding human and other mammalian insulin cDNA sequences as well as the function of the claimed sequences. However, neither the specification nor the general knowledge of those skilled in the art provide evidence of any partial structure which would be expected to be common to the members of the genus. Moreover, there is post filing date evidence that indicates that there is a lack of a structural relationship between the rat insulin cDNA sequences and other mammalian insulin cDNA sequences. In view of the above considerations one of skill in the art would not recognize that applicant was in possession of the necessary common features or attributes possessed by members of the genus, because rat cDNA sequences are not representative of the claimed genus. Consequently, since applicant was in

possession only of the rat insulin cDNA and since the art recognized variation among the species of the genus of cDNAs that encode mammalian insulin, the rat insulin cDNA was not representative of the claimed genus. Therefore, the applicant was not in possession of the genus of mammalian insulin cDNAs as encompassed by claim 1.

Conclusion:

Claims 1 and 2 do not meet the written description requirement.

Example 18: <u>Process claim where the novelty is in the method steps.</u>

Specification: The specification teaches a method for producing proteins using mitochondria from the fungus *Neurospora crassa*. In the method, mitochondria are isolated from this fungus and transformed with a mitochondrial expression vector which comprises a nucleic acid encoding a protein of interest. The protein is subsequently expressed, the mitochondria is lysed, and the protein is isolated. The specification exemplifies the expression of β-galactosidase using the claimed method using a cytochrome oxidase promoter.

Claim:

1. A method of producing a protein of interest comprising;

obtaining *Neurospora crassa* mitochondria,

transforming said mitochondria with a expression vector comprising a nucleic acid that encodes said protein of interest,

expressing said protein in said mitochondria, and

recovering said protein of interest.

Analysis:

A review of the specification reveals that *Neurospora crassa* mitochondrial gene expression is essential to the function/operation of the claimed invention. A particular nucleic acid is not essential to the claimed invention.

A search of the prior art reveals that the claimed method of expression in *Neurospora crassa* is novel and unobvious.

The claim is drawn to a genus, i.e., any of a variety of methods that can be used for expressing protein in the mitochondria.

There is actual reduction to practice of a single embodiment, i.e., the expression of β-galactosidase.

The art indicates that there is no substantial variation within the genus because there are a limited number of ways to practice the process steps of the claimed invention.

The single embodiment is representative of the genus based on the disclosure of *Neurospora crassa* mitochondria as a gene expression system, considered along with the level of skill and knowledge in the gene expression art. One of skill in the art would recognize that applicant was in possession of all of the various expression methods necessary to practice the claimed invention.

Conclusion:

The claimed invention is adequately described.

2 G

Molecular Cloning

A LABORATORY MANUAL

SECOND EDITION

J. Sambrook
UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL CENTER

E.F. Fritsch
GENETICS INSTITUTE

T. Maniatis
HARVARD UNIVERSITY

Cold Spring Harbor Laboratory Press
1989

IDENTIFICATION OF cDNA CLONES OF INTEREST

Methods of Screening

There are three methods to screen cDNA libraries for clones of interest:

- Nucleic acid hybridization
- Immunological detection of specific antigens
- Sib selection either by hybrid selection and translation of mRNA or by production of biologically active molecules

Most cloning projects today are aimed at isolating cDNAs corresponding to rare mRNAs and therefore require screening of large numbers of recombinant clones. This can be carried out effectively with only two types of reagents: antibodies and nucleic acid probes. In those rare instances when both types of reagents are available, nucleic acid probes are preferred because they can be used under a variety of different stringencies that minimize the chance of undesirable cross-reactions. Furthermore, nucleic acid probes will detect all clones that contain cDNA sequences, whereas antibodies will react only with a subset of these clones (in some cases one in six at best) in which the cDNA has been inserted into the vector in the correct reading frame and orientation. cDNA libraries that are to be screened by antibodies therefore need to be larger (by a factor of at least 6) than those that are to be screened by nucleic acid probes. Consequently, when using antibody probes to search for a cDNA clone corresponding to a mammalian mRNA present at the level of 1 molecule/cell or less, it is desirable to construct cDNA expression libraries that contain in excess of 10^7 members. This is not easy, especially when the amounts of mRNA are limited. Furthermore, screening a library of this size is expensive and laborious, and it becomes worthwhile to explore methods to enrich the mRNA (or cDNA derived from it) for the sequences of interest (see pages 8.6–8.10).

NUCLEIC ACID HYBRIDIZATION

This is the most commonly used and reliable method of screening cDNA libraries for clones of interest. None of the other methods displays such an abundance of attractive features. Screening by nucleic acid hybridization allows extremely large numbers of clones to be analyzed simultaneously and rapidly, does not require that the cDNA clones be full-length, and does not require that an antigenically or biologically active product be synthesized in the host cell. Furthermore, as a result of more than 20 years of work, the theoretical basis of nucleic acid hybridization is well-understood. This has led to the development of a large number of different techniques that can accommodate nucleic acid probes of very different lengths and specificities. Details of the methods for the preparation and use of these probes are presented in Chapters 10 and 11.

Homologous probes

Homologous probes contain at least part of the exact nucleic acid sequence of the desired cDNA clone. They are used in a variety of circumstances, for

example, when a partial clone of an existing cDNA is used to isolate a full-length clone from a cDNA library. Usually, a fragment derived from one end or the other of the existing clone is isolated, radiolabeled in vitro, and used to probe a library. Hybridization with homologous probes is always carried out under stringent conditions.

Partially homologous probes

Partially homologous probes are used to detect cDNA clones that are related, but not identical, to the probe sequences. If neither antibody nor nucleic acid probes are available, a number of alternative strategies can be considered. For example, if the same gene has already been cloned from another species or if a related gene has been cloned from the same species, it would be worthwhile carrying out a series of trial experiments to determine whether there is sufficient conservation of nucleic acid sequence to allow the screening of a cDNA library by hybridization. This is most easily accomplished by performing a series of Southern and northern hybridizations at different stringencies. For example, a large batch (50 μg) of genomic DNA is cleaved with a restriction enzyme that cleaves the probe sequence at one or two well-separated sites. It is a good idea to digest an equal amount of genomic DNA of the original species for use as a positive control. Aliquots (5–10 μg) of the digests are then applied to adjacent slots of a 0.8% agarose gel, electrophoresis is carried out, and the fragments are then transferred to a nitrocellulose filter as described in Chapter 9, pages 9.34–9.41. The filter is cut into strips, each of which is hybridized under different conditions to identical amounts of radioactive probe. For aqueous hybridization, the ionic strength of the solution is kept constant (usually 1 M Na^+) while the temperature of annealing is progressively lowered (from 68°C to 42°C). The strips are then washed extensively at the temperature of hybridization with a solution containing 2 × SSC, 0.5% SDS. When hybridization is carried out in solvents containing formamide, the temperature and ionic strength are usually kept constant (42°C and 6 × SSC [or 6 × SSPE], respectively) while the amount of formamide in the annealing buffer is progressively lowered from 50% to 0%. The strips are then washed extensively at 50°C in 6 × SSC, 0.5% SDS. A similar series of hybridizations can be carried out with mRNA preparations that have been fractionated by electrophoresis and transferred to a solid support. In both cases, the aim is to establish conditions that will allow the previously cloned gene to be used as a probe for the cDNA of interest, without undue interference from background hybridization.

Total cDNA probes

Total cDNA probes are prepared by uniform incorporation of radiolabeled nucleotides with reverse transcriptase or end-labeling of total or fractionated poly(A)$^+$ mRNA. They can be used to screen libraries of cDNA for specific clones *if* the cDNA clones of interest correspond to mRNA species present in the initial population at a frequency of at least 1 in 200 (see Gergen et al. 1979; Dworkin and Dawid 1980). It is not possible to detect cDNA clones homologous to species that are represented rarely in the mRNA preparation.

Subtracted cDNA probes

Subtracted cDNA probes are often used to probe cDNA libraries for clones that correspond to mRNAs that are differentially regulated. A cDNA probe prepared from one type of mRNA is depleted of sequences that are present in a second type of mRNA by subtractive hybridization (Timberlake 1980; Zimmerman et al. 1980). Typically, the cDNA is hybridized two or three times in succession to a 20-fold excess of the second mRNA, and the cDNA:mRNA hybrids are removed by chromatography on hydroxyapatite. The unhybridized cDNA is then annealed to a 100-fold excess of the mRNA preparation from which it was originally synthesized, and the resulting cDNA:mRNA hybrids are this time recovered by chromatography on hydroxyapatite. After the mRNA is removed by alkaline hydrolysis, the cDNA, which is highly enriched for sequences specific to the original mRNA, is used to probe a cDNA library for clones homologous to these sequences.

Subtracted cDNA probes are particularly valuable when there are very few differences between the two starting mRNA preparations, i.e., when most species of mRNA are represented equally in the two preparations and a small proportion (<2%) of the mRNAs are not present at all in one preparation. cDNAs that have been cloned using subtracted cDNA probes include the murine J immunoglobulin chain (Mather et al. 1981) and the murine T-cell receptor (Hedrick et al. 1984).

A slightly different approach is used when two preparations of mRNA share sequences that are present at different concentrations. Examples of such sib pairs might be mRNAs extracted from control cells and cells that have been exposed to heat shock, drugs, or hormones. cDNAs corresponding to mRNAs whose expression is altered by such treatments can often be detected by *differential hybridization*. ^{32}P-labeled first-strand cDNAs are synthesized in vitro using both mRNAs as templates. Most of the cDNA sequences correspond to mRNAs whose concentrations are not appreciably changed by the treatment to which the cells were exposed. However, a minority of the cDNAs will be copied from mRNAs whose concentrations are significantly increased or decreased. The two cDNA probes are then used to screen replicas of a cDNA library constructed from mRNA extracted from control cells (when searching for mRNAs that are repressed) or treated cells (when searching for mRNAs that are induced). The clones that hybridize preferentially to one of the cDNA probes are chosen for further analysis. Among the many examples of genes cloned in this way are the galactose-inducible genes of yeast (St. John and Davis 1979), human fibroblast interferon (Taniguchi et al. 1980), the glucose-regulated proteins of mammalian cells (Lee et al. 1981), growth-related proteins (Foster et al. 1982; Cochran et al. 1983; Linzer and Nathans 1983), differentiation-specific proteins (Spiegelman et al. 1983), and a variety of heat-shock proteins and stress proteins (see, e.g., Mason et al. 1986). The procedure has also been used to identify cDNA clones of developmentally regulated mRNAs from organisms of many different species including *Xenopus* (Williams and Lloyd 1979; Weeks et al. 1985), *Dictyostelium* (Rowekamp and Firtel 1980; Mehdy et al. 1983), sea urchins (Lasky et al. 1980), and mice (Gorman et al. 1985).

Synthetic oligonucleotide probes

Synthetic oligonucleotide probes are tracts of dNTPs of defined sequence that have been synthesized in vitro. The sequence of these probes is deduced, using the genetic code, from short regions of the known amino acid sequence of the protein of interest. Because of the degeneracy of the genetic code, it is very unlikely that a given sequence of amino acids will be specified by a predictable single oligonucleotide of defined sequence. Instead, in the vast majority of cases, the same sequence of amino acids can be specified by many different oligonucleotides. There is no way to know with certainty which of these oligonucleotides is actually used in the gene of interest. Three solutions have been found to this problem:

1. A family of oligonucleotides can be synthesized containing all possible sequences that can code for a given sequence of amino acids. The number of members in this family depends on the degree of degeneracy of the codons for the particular amino acids. However, since all possible oligonucleotide sequences are represented, at least one of the members will match perfectly with the cDNA clone of interest. To keep the size of each family within manageable proportions, short oligonucleotides (14–17 nucleotides) are generally used—the minimum size that is practical for hybridization. Often, more than one family of oligonucleotides is synthesized based on separate sequences of amino acids.
2. A longer (40–60-base) oligonucleotide of unique sequence can be synthesized using the most commonly used codon for each amino acid. (Avoid using the dinucleotide CpG, since it is underrepresented in most eukaryotic DNAs.) Almost certainly, this oligonucleotide will not match exactly the sequence in the cDNA, but it will fit well enough to be detected by hybridization under nonstringent conditions.
3. An oligonucleotide can be synthesized that contains a base such as inosine at positions of high potential degeneracy. Inosine can pair with all four conventional bases without seriously compromising the stability of the resulting hybrid. It is therefore possible to generate families of longer oligonucleotides that are reduced in number and yet are capable of hybridizing to virtually all cDNA clones that are likely to code for the protein of interest.

Finally, if the protein sequence available is from the amino terminus of the protein, the cDNA library that is to be screened must be of high quality to ensure that most of the 5′ terminus of the mRNA is represented. For a detailed discussion of synthetic oligonucleotide probes, see Chapter 11.

IMMUNOLOGICAL DETECTION OF SPECIFIC ANTIGENS

cDNA libraries constructed in expression vectors such as λgt11, λgt18–23, λZAP, and λORF8 can be screened with antibody directed against the protein of interest (see Chapter 12 for experimental details). Nitrocellulose filters imprinted with the detritus of bacterial lysis are soaked in a solution

containing the antibody. After washing, the filter is incubated with *Staphylococcus aureus* protein A or with a second antibody directed against the species-specific epitopes of the first antibody. In the original descriptions of the method, the secondary ligand was radiolabeled with ^{125}I. Today, the secondary ligand is covalently linked to an enzyme whose activity can be detected histochemically (e.g., alkaline phosphatase).

The key to success with this method lies in the quality of the antibody. It is essential that the antibody efficiently recognize the denatured protein (i.e., it should produce strong signals on western blots). Screening is made easier and more sensitive if, in addition, the antibody is derived from a polyclonal antiserum of high titer. Because such antisera normally react with many different epitopes, the chances of detecting a cDNA clone that expresses a fragment of the protein of interest are increased. Polyclonal antisera often contain cross-reacting antibodies that recognize nonrecombinant components of the bacterial lysate; these must be removed before screening is undertaken (see Chapter 12).

The background of nonspecific binding is much lower when a monoclonal antibody is used as a probe. However, the number of recombinants that can be detected is also reduced, because each individual monoclonal antibody can react with only a single epitope. The ideal immunological probe might therefore consist of a cocktail of several different monoclonal antibodies, each of which reacts strongly with denatured protein.

SIB SELECTION OF cDNA CLONES

Two methods of screening are based on the concept of dividing a large cDNA library into a manageable number of pools, each consisting of between 10 and 100 clones. These pools are then tested for the sequence of interest. The lower complexity of each pool allows methods that are relatively insensitive to be applied to a complete cDNA library. After a pool is identified that scores positively, it is subdivided into successively smaller and smaller pools, each of which is retested until the cDNA clone of interest is isolated. The process of sib selection and analysis can sometimes be performed more rapidly if, during subdivision of the initial pool, the individual clones are assigned to their subpools in a matrix format (Wong et al. 1985). This expands the number of subpools by a factor of 2 or 3, but it often leads directly to the identification of the clone of interest.

Hybrid selection

In this method, cDNA clones carrying sequences complementary to specific mRNAs are denatured, immobilized on a solid matrix, and hybridized to preparations of mRNA. The mRNA:cDNA hybrids are then heated to release the mRNA, which is then translated in a cell-free protein-synthesizing system or in *Xenopus* oocytes. The translation products are identified by immunoprecipitation and/or SDS-polyacrylamide gel electrophoresis (see Chapter 18).

Today, this procedure is used mainly to confirm the identification of cDNA clones isolated by other means. However, before the development of methods to screen cDNA libraries with immunological or oligonucleotide probes, it was

also used occasionally for the primary isolation of cDNA clones. For example, Parnes et al. (1981), using DNA from pools that contained as many as 100 individual cDNAs cloned in plasmids, were able to detect specific translation of an mRNA that represented approximately 0.03% of the total cellular mRNA. This entire procedure is labor-intensive and is worth attempting only as a last resort with mRNAs that can be greatly enriched by size selection and for which no other probes are available.

Production of biologically active molecules

A few groups have used sib selection and analysis to screen cDNA libraries for the production of biologically active protein molecules in cells. For example, in vitro assays have been used to identify cDNA clones that express the human lymphokines colony-stimulating factor and interleukin-3 in cultures of mammalian cells (Wong et al. 1985; Yang et al. 1986). This approach is usually undertaken when no other methods of screening are available and when the protein product is small enough to give reasonable assurance that the cDNA library will contain full-length clones.

Methods to Validate Clones of cDNA

cDNA libraries are usually plated at high density for screening with antibody or nucleic acid probes, and any clones that react positively in the first round require several additional cycles of plating and screening before they can be considered pure. However, the ability to react consistently with a particular probe, although an encouraging and necessary property, is not sufficient to prove that a given cDNA clone is derived from the mRNA of interest. The only absolute proof of identity is to show that the cDNA clone contains an open reading frame that codes for the entire amino acid sequence of the protein. Since this is clearly impractical for most proteins of current interest, other, less elemental tests must be used. In decreasing order of rigor, these include:

- Expression from the full-length cDNA in prokaryotic or eukaryotic cells of a protein that displays the correct biological or enzymatic activity.
- Correspondence between portions of the nucleotide sequence of the cDNA and the amino acid sequences of peptides derived from the purified protein.
- Correspondence between the peptide maps of the polypeptide synthesized in vitro by transcripts of the cDNA clone and peptide maps of the authentic protein.
- Immunoprecipitation of the polypeptide synthesized in vitro or in vivo from transcripts of the cDNA clone by antibodies raised against the protein of interest. The stringency of this test increases when it is carried out with a series of monoclonal antibodies that recognize different epitopes on the protein.
- Immunoprecipitation of the authentic protein with antibodies raised against synthetic peptides whose sequences are determined by the nucleic acid sequence of the cloned cDNA.

If current estimates are correct, we shall move in the short span of 50 years from the discovery of the structure of DNA to the elucidation of the complete sequence of the human genome. Although this rapid rate of progress has been catalyzed by many theoretical and technical advances, none has been so informative as the ability to construct libraries of eukaryotic genomic DNA. Originally devised as banks from which individual bacterial genes could be isolated and analyzed (Clarke and Carbon 1976), genomic DNA libraries are now increasingly viewed as matrices that can be assembled into maps of the genome from which they were originally derived.

The feasibility of using libraries in this way was established in the late 1970s, when Maniatis et al. (1978) devised a strategy to generate and clone large numbers of random fragments of mammalian genomic DNA. Before then, genomic libraries consisted of fragments of DNA obtained by complete digestion of genomic DNA with a restriction enzyme such as *Eco*RI. The earliest libraries of mammalian genomic DNA contained only subsets of this large population of fragments because it was necessary to enrich for the sequences of interest due to the comparative inefficiency of packaging of bacteriophage λ DNA. For example, Tilghman et al. (1977) constructed a library from fragments of mouse DNA that had been enriched approximately tenfold for β-globin sequences by preparative gel electrophoresis and reversed-phase chromatography (Hardies and Wells 1976). The improvement of in vitro packaging systems (Hohn and Murray 1977; Sternberg et al. 1977) and the development of in situ hybridization techniques (Benton and Davis 1977) soon allowed much larger libraries to be constructed from unfractionated populations of restriction fragments (Maniatis et al. 1978; Smithies et al. 1978), thus eliminating the need to enrich the starting DNA for the sequences of interest. The inherent limitations of genomic DNA libraries constructed from complete digests of genomic DNA with a restriction enzyme such as *Eco*RI then quickly became apparent. These limitations include the following:

- Because of their large size, libraries of complete digests are laborious both to create and to screen. The average distance between *Eco*RI sites in the mammalian genome is approximately 4 kb (Botchan et al. 1974), and complete digests therefore contain approximately 10^6 different fragments of DNA. Consequently, a library constructed from these fragments needs to contain almost 10^7 independent recombinants to have a reasonable chance of including a desired sequence.
- If the sequence of interest contains one or more recognition sites for the particular restriction enzyme, it will be cloned in two or more nonoverlapping recombinants. In libraries of complete restriction digests, there is no way to "walk" from one recombinant clone to another that contains the neighboring restriction fragment in the original genomic DNA.
- Because of the quasi-random distribution of restriction sites in mammalian DNA, the sequence of interest may by chance be located on a fragment of genomic DNA that is too large or too small for the vector to accept.

The solution to these problems was to clone large pieces of DNA generated

either by mechanical shearing (Wensink et al. 1974; Clarke and Carbon 1976) or by partial digestion with restriction enzymes that cleave the target DNA frequently (Maniatis et al. 1978). Unfortunately, the efficiency of cloning sheared DNA was not sufficient to obtain representative libraries. Thus, virtually all of the libraries used to isolate single-copy genes were produced by partial digestion of genomic DNA with restriction enzymes. These DNA fragments were fractionated by density gradient centrifugation or preparative gel electrophoresis to select those whose size was suitable for insertion into the vector (Maniatis et al. 1978). This method, which is used in an essentially unchanged form today, helps to ensure that there is no systematic exclusion of sequences from the cloned library merely because of an unfortunate distribution of restriction sites. The following advantages are gained by constructing libraries of randomly cleaved DNA:

- *The ability to "walk" along the eukaryotic chromosome in a way that is impossible with a library of nonoverlapping restriction fragments.* Random cleavage of genomic DNA generates collections of overlapping fragments from each chromosome. Thus, a library of these fragments should consist of 46 independent collections of overlapping clones. If the library contains no gaps, the clones forming an individual series will correspond to the entire DNA sequence of an individual chromosome. To walk along a chromosome, a segment of nonrepetitive DNA derived from one end of a given recombinant is used as a probe to identify overlapping clones that contain the adjacent sequence. This process can be repeated until clones have been identified that cover the entire region of interest.
- *The ability to isolate segments of DNA without knowledge of the location of restriction sites in and around the target sequences.*
- *A reduction in the size of the library.* Because the DNA fragments selected for cloning are large, the number of recombinants required to generate a mammalian genomic DNA library is significantly reduced.

Note: The exact probability of having any given DNA sequence in the library can be calculated from the equation

$$N = \frac{\ln(1-P)}{\ln(1-f)}$$

where P is the desired probability, f is the fractional proportion of the genome in a single recombinant, and N is the necessary number of recombinants (Clarke and Carbon 1976). For example, to achieve a 99% probability ($P = 0.99$) of having a given DNA sequence represented in a library of 17-kb fragments of a mammalian genome (3×10^9 bp)

$$N = \frac{\ln(1-0.99)}{\ln\left(1-\left[\frac{1.7\times10^4}{3\times10^9}\right]\right)} = 8.1\times10^5$$

an asserted utility, unless countervailing evidence can be provided that shows that one of ordinary skill in the art would have a legitimate basis to doubt the credibility of such a statement. Similarly, Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered.

Once a *prima facie* showing of no specific and substantial credible utility has been properly established, the applicant bears the burden of rebutting it. The applicant can do this by amending the claims, by providing reasoning or arguments, or by providing evidence in the form of a declaration under 37 CFR 1.132 or a patent or a printed publication that rebuts the basis or logic of the *prima facie* showing. If the applicant responds to the *prima facie* rejection, the Office personnel should review the original disclosure, any evidence relied upon in establishing the *prima facie* showing, any claim amendments, and any new reasoning or evidence provided by the applicant in support of an asserted specific and substantial credible utility. It is essential for Office personnel to recognize, fully consider and respond to each substantive element of any response to a rejection based on lack of utility. Only where the totality of the record continues to show that the asserted utility is not specific, substantial, and credible should a rejection based on lack of utility be maintained.

If the applicant satisfactorily rebuts a *prima facie* rejection based on lack of utility under § 101, withdraw the § 101 rejection and the corresponding rejection imposed under § 112, first paragraph.

Dated: December 29, 2000.

Q. Todd Dickinson,

Under Secretary of Commerce for Intellectual Property and Director of the United States Patent and Trademark Office.

[FR Doc. 01–322 Filed 1–4–01; 8:45 am]

BILLING CODE 3510–16–U

DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

[Docket No. 991027288–0264–02]

RIN 0651–AB10

Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶1, "Written Description" Requirement

AGENCY: United States Patent and Trademark Office, Commerce.

ACTION: Notice.

SUMMARY: These Guidelines will be used by USPTO personnel in their review of patent applications for compliance with the "written description" requirement of 35 U.S.C. 112, ¶ 1. These Guidelines supersede the "Revised Interim Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 'Written Description' Requirement" that were published in the **Federal Register** at 64 FR 71427, Dec. 21, 1999, and in the Official Gazette at 1231 O.G. 123, Feb. 29, 2000. These Guidelines reflect the current understanding of the USPTO regarding the written description requirement of 35 U.S.C. 112, ¶ 1, and are applicable to all technologies.

DATES: The Guidelines are effective as of January 5, 2001.

FOR FURTHER INFORMATION CONTACT: Stephen Walsh by telephone at (703) 305–9035, by facsimile at (703) 305–9373, by mail to his attention addressed to United States Patent and Trademark Office, Box 8, Washington, DC 20231, or by electronic mail at "stephen.walsh@uspto.gov"; or Linda Therkorn by telephone at (703) 305–8800, by facsimile at (703) 305–8825, by mail addressed to Box Comments, Commissioner for Patents, Washington, DC 20231, or by electronic mail at "linda.therkorn@uspto.gov."

SUPPLEMENTARY INFORMATION: As of the publication date of this notice, these Guidelines will be used by USPTO personnel in their review of patent applications for compliance with the "written description" requirement of 35 U.S.C. 112, ¶ 1. Because these Guidelines only govern internal practices, they are exempt from notice and comment rulemaking under 5 U.S.C. 553(b)(A).

Discussion of Public Comments

Comments were received from 48 individuals and 18 organizations in response to the request for comments on the "Revised Interim Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 'Written Description' Requirement" published in the **Federal Register** at 64 FR 71427, Dec. 21, 1999, and in the Official Gazette at 1231 O.G. 123, Feb. 29, 2000. The written comments have been carefully considered.

Overview of Comments

The majority of comments favored issuance of final written description guidelines with minor revisions. Comments pertaining to the written description guidelines are addressed in detail below. A few comments addressed particular concerns with respect to the associated examiner training materials that are available for public inspection at the USPTO web site (www.uspto.gov). Such comments will be taken under advisement in the revision of the training materials; consequently, these comments are not specifically addressed below as they do not impact the content of the Guidelines. Several comments raised issues pertaining to the patentability of ESTs, genes, or genomic inventions with respect to subject matter eligibility (35 U.S.C. 101), novelty (35 U.S.C. 102), or obviousness (35 U.S.C. 103). As these comments do not pertain to the written description requirement under 35 U.S.C. 112, they have not been addressed. However, the aforementioned comments are fully addressed in the "Discussion of Public Comments" in the "Utility Examination Guidelines" Final Notice, which will be published at or about the same time as the present Guidelines.

Responses to Specific Comments

(1) *Comment:* One comment stated that the Guidelines instruct the patent examiner to determine the correspondence between what applicant has described as the essential identifying characteristic features of the invention and what applicant has claimed, and that such analysis will lead to error. According to the comment, the examiner may decide what applicant should have claimed and reject the claim for failure to claim what the examiner considers to be the invention. Another comment suggested that the Guidelines should clarify what is meant by "essential features of the invention." Another comment suggested that what applicant has identified as the "essential distinguishing characteristics" of the invention should be understood in terms of *Fiers* v. *Revel*, 984 F.2d 1164, 1169, 25 USPQ2d 1601, 1605 (Fed. Cir. 1993) ("Conception of a substance claimed *per se* without reference to a process requires conception of its structure, name,

formula, or definitive chemical or physical properties.").

Response: The suggestions have been adopted in part. The purpose of the written description analysis is to confirm that applicant had possession of what is claimed. The Guidelines have been modified to instruct the examiners to compare the scope of the invention claimed with the scope of what applicant has defined in the description of the invention. That is, the Guidelines instruct the examiner to look for consistency between a claim and what provides adequate factual support for the claim as judged by one of ordinary skill in the art from reading the corresponding written description.

(2) *Comment:* Two comments urge that *Regents of the University of California* v. *Eli Lilly & Co.*, 119 F.3d 1559, 43 USPQ2d 1398 (Fed. Cir. 1997), is bad law and should not be followed by the USPTO because it conflicts with binding precedent, such as *Vas-Cath* v. *Mahurkar*, 935 F.2d 1555, 19 USPQ2d 1111 (Fed. Cir. 1991). *Response:* The final Guidelines are based on the Office's current understanding of the law and are believed to be fully consistent with binding precedent of the U.S. Supreme Court and the U.S. Court of Appeals for the Federal Circuit. *Eli Lilly* is a precedential decision by the Court that has exclusive jurisdiction over appeals involving patent law. Accordingly, the USPTO must follow *Eli Lilly*. Furthermore, the USPTO does not view *Eli Lilly* as conflicting with *Vas-Cath*. *Vas-Cath* explains that the purpose of the written description requirement is to ensure that the applicant has conveyed to those of skill in the art that he or she was in possession of the claimed invention at the time of filing. *Vas-Cath*, 935 F.2d at 1563–64, 19 USPQ2d at 1117. *Eli Lilly* explains that a chemical compound's name does not necessarily convey a written description of the named chemical compound, particularly when a genus of compounds is claimed. *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1405. The name, if it does no more than distinguish the claimed genus from all others by function, does not satisfy the written description requirement because "it does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus." *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406. Thus, *Eli Lilly* identified a set of circumstances in which the words of the claim did not, without more, adequately convey to others that applicants had possession of what they claimed.

(3) *Comment:* Several comments urged that the Guidelines do not recognize the inconsistency between the original claim doctrine and the written description requirement as set out in *Fiers* and *Eli Lilly*. On the other hand, another comment asserts that there is no strong presumption that an originally filed claim constitutes an adequate written description of the claimed subject matter. Several comments indicate that *in haec verba* support should be sufficient to comply with the written description requirement. Two comments urge that the concept of constructive reduction to practice upon filing of an application has been ignored. *Response:* As noted above, the USPTO does not find *Fiers* and *Eli Lilly* to be in conflict with binding precedent. An original claim may provide written description for itself, but it still must be an *adequate* written description which establishes that the inventor was in possession of the invention. The "original claim doctrine" is founded on cases which stand for the proposition that originally filed claims are part of the written description of an application as filed, and thus subject matter which is present only in originally filed claims need not find independent support in the specification. *See, e.g., In re Koller*, 613 F.2d 819, 824, 204 USPQ 702, 706 (CCPA 1980) (later added claims of similar scope and wording were adequately described by original claims); *In re Gardner*, 480 F.2d 879, 880, 178 USPQ 149, 149 (CCPA 1973) ("Under these circumstances, we consider the original claim in itself adequate 'written description' of the claimed invention. It was equally a 'written description' * * * whether located among the original claims or in the descriptive part of the specification."). However, as noted in the preceding comment, *Eli Lilly* identified a set of circumstances in which the words of the claim did not, without more, adequately convey to others that applicants had possession of what they claimed. When the name of a novel chemical compound does not convey sufficient structural information about the compound to identify the compound, merely reciting the name is not enough to show that the inventor had possession of the compound at the time the name was written. The Guidelines indicate that there is a "strong presumption" that an adequate written description of the claimed invention is present when the application is filed, consistent with *In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976) ("we are of the opinion that the PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims."). In most cases, the statement that "an originally filed claim is its own written description," is borne out because the claim language conveys to others of skill in the art that the applicant was "in possession" of what is claimed. The Guidelines emphasize that the burden of proof is on the examiner to establish that a description as filed is not adequate and require the examiner to introduce sufficient evidence or technical reasoning to shift the burden of going forward with contrary evidence to the applicant.

(4) *Comment:* One comment stated that the Guidelines change the substance of the written description requirement to require some level of enablement. The comment stated that the *Eli Lilly* case should not be followed because its change in the quality of the description required is in conflict with precedent. Another comment suggested that to comply with the written description requirement, the description must both (i) demonstrate possession of the claimed invention by the applicant; and (ii) put the public in possession of the claimed invention. *Response:* As noted in the comment above, the USPTO is bound by the Federal Circuit's decision in *Eli Lilly*. The Guidelines have been revised to clarify that an applicant must provide a description of the claimed invention which shows that applicant was in possession of the claimed invention. The suggestion to emphasize that the written description requirement must put the public in possession of the invention has not been adopted because it removes much of the distinction between the written description requirement and the enablement requirement. Although the two concepts are entwined, they are distinct and each is evaluated under separate legal criteria. The written description requirement, a question of fact, ensures that the inventor conveys to others that he or she had possession of the claimed invention; whereas, the enablement requirement, a question of law, ensures that the inventor conveys to others how to make and use the claimed invention.

(5) *Comment:* One comment suggested that the Guidelines should provide examples of situations in which the written description requirement was met but the enablement requirement was not, and vice versa. Another comment stated that examiners often use enablement language in making

written description rejections.

Response: The enablement and written description requirements are not coextensive and, therefore, situations will arise in which one requirement is met but the other is not. Federal Circuit case law demonstrates many circumstances where enablement or written description issues, but not both, were before the Court. These Guidelines are intended to clarify for the examining corps the criteria needed to satisfy the written description requirement. For examples applying these Guidelines to hypothetical fact situations, see the "Synopsis of Application of Written Description Guidelines" (examiner training materials available on-line at *http://www.uspto.gov/web/menu/written.pdf).* These examples, as well as the examination form paragraphs and instructions on their proper use, provide the appropriate language examiners should use in making written description rejections.

(6) *Comment:* One comment disagreed with the statement in an endnote that "the fact that a great deal more than just a process is necessary to render a product invention obvious means that a great deal more than just a process is necessary to provide written description for a product invention." The comment indicated that the statement is overly broad and inconsistent with the "strong presumption that an adequate written description of the claimed invention is present when the application is filed." As an extreme case, for example, for product-by-process claims, nothing else would be needed to provide the written description of the product. *Response:* The endnote has been clarified and is now more narrowly drawn. However, there is no *per se* rule that disclosure of a process is sufficient to adequately describe the products produced by the process. In fact, *Fiers* v. *Revel* and *Eli Lilly* involved special circumstances where the disclosure of a process of making and the function of the product alone did not provide an adequate written description for product claims. Even when a product is claimed in a product-by-process format, the adequacy of the written description of the process to support product claims must be evaluated on a case-by-case basis.

(7) *Comment:* Several comments urge that actual reduction to practice, as a method of satisfying the written description requirement by demonstrating possession, has been over-emphasized. *Response:* The Guidelines have been clarified to state that describing an actual reduction to practice is one of a number of ways to show possession of the invention. Description of an actual reduction to practice offers an important "safe haven" that applies to all applications and is just one of several ways by which an applicant may demonstrate possession of the claimed invention. Actual reduction to practice may be crucial in the relatively rare instances where the level of knowledge and level of skill are such that those of skill in the art cannot describe a composition structurally, or specify a process of making a composition by naming components and combining steps, in such a way as to distinguish the composition with particularity from all others. Thus, the emphasis on actual reduction to practice is appropriate in those cases where the inventor cannot provide an adequate description of what the composition is, and a definition by function is insufficient to define a composition "because it is only an indication of what the [composition] does, rather than what it is." *Eli Lilly,* 119 F.3d at 1568, 43 USPQ at 1406. *See also Amgen Inc.* v. *Chugai Pharmaceutical Co.,* 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991).

(8) *Comment:* One comment asserts that the citation to *Pfaff* v. *Wells Electronics, Inc.,* 525 U.S. 55, 48 USPQ2d 1641 (1998) is inappropriate and should be deleted because *Pfaff* is concerned with § 102(b) on-sale bar, not written description. Another comment suggested that the Guidelines should provide an explanation of how the "ready for patenting" concept of *Pfaff* should be used in determining compliance with the written description requirement. *Response:* The Guidelines state the general principle that actual reduction to practice is not required to show possession of, or to adequately describe, a claimed invention (although, as noted in the previous comment, an actual reduction to practice is crucial in relatively rare instances). An alternative is to show that the invention described was "ready for patenting" as set out in *Pfaff.* For example, a *description* of activities that demonstrates the invention was "ready for patenting" satisfies the written description requirement. As *Wertheim* indicates, "how the specification accomplishes this is not material." 541 F.2d at 262, 191 USPQ at 96.

(9) *Comment:* One comment stated that the written description of a claimed DNA should be required to include the complete sequence of the DNA and claims should be limited to the DNA sequence disclosed. *Response:* Describing the complete chemical structure, *i.e.,* the DNA sequence, of a claimed DNA is one method of satisfying the written description requirement, but it is not the only method. *See Eli Lilly,* 119 F.3d at 1566, 43 USPQ2d at 1404 ("An adequate written description of a DNA * * * requires a precise definition, *such as* by structure, formula, chemical name; or physical properties." (emphasis added, internal quote omitted)). Therefore, there is no basis for a *per se* rule requiring disclosure of complete DNA sequences or limiting DNA claims to only the sequence disclosed.

(10) *Comment:* One comment stated that it is difficult to envision how one could provide a description of sufficient identifying characteristics of the invention without physical possession of a species of the invention, and thus this manner of showing possession should be considered as a way to show actual reduction to practice. *Response:* This suggestion has not been adopted. The three ways of demonstrating possession as set forth in the Guidelines are merely exemplary and are not mutually exclusive. While there are some cases where a description of sufficient relevant identifying characteristics will evidence an actual reduction to practice, there are other cases where it will not. *See, e.g., Ralston Purina Co.* v. *Far-Mar-Co, Inc.,* 772 F.2d 1570, 1576, 227 USPQ 177, 180 (Fed. Cir. 1985) (disclosure taken with the knowledge of those skilled in the art may be sufficient support for claims).

(11) *Comment:* One comment stated that the Guidelines should be revised to indicate that the test of disclosure of sufficiently detailed drawings should be expanded to include structural claiming of chemical entities. *Response:* The suggestion has been adopted.

(12) *Comment:* One comment stated that the Guidelines should reflect that an inventor is in possession of the invention when the inventor demonstrably has at least a complete conception thereof, and that factors and attributes which provide proof of written description should include evidence typically provided to prove a complete conception. *Response:* The suggestion has not been adopted because the conception analysis typically involves documentary evidence in addition to the description of the invention in the application as filed. However, it is acknowledged that if evidence typically provided to prove a complete conception is present in the specification as filed, it would be sufficient to show possession. The Federal Circuit has stated "[t]he conception analysis necessarily turns on the inventor's ability to describe his invention with particularity. Until he can do so, he cannot prove possession

of the complete mental picture of the invention." *Burroughs Wellcome Co.* v. *Barr Labs., Inc.*, 40 F.3d 1223, 1228, 32 USPQ2d 1915, 1919 (Fed. Cir. 1994). As further noted by the Federal Circuit, in order to prove conception, "a party must show possession of every feature recited in the count, and that every limitation of the count must have been known to the inventor at the time of the alleged conception." *Coleman* v. *Dines*, 754 F.2d 353, 359, 224 USPQ 857, 862 (Fed. Cir. 1985).

(13) *Comment:* One comment indicated that a "possession" test does not appear in Title 35 of the U.S. Code and is not clearly stated by the Federal Circuit. Therefore, it is recommended that patent examiners be directed to use existing judicial precedent to make rejections of claims unsupported by a statutory written description requirement. *Response:* While the Federal Circuit has not specifically laid out a "possession" test, the Court has clearly indicated that possession is a cornerstone of the written description inquiry. *See, e.g., Vas-Cath, Inc.* v. *Mahurkar*, 935 F.2d 1555, 1563, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991); *see also Purdue Pharma L.P.* v. *Faulding Inc.*, 230 F.3d 1320, 1323, 56 USPQ2d 1481, 1483 (Fed. Cir. 2000) ("[o]ne skilled in the art, reading the disclosure, must immediately discern the limitation at issue in the claims") (internal quote omitted). The possession test as set forth in the Guidelines is extrapolated from case law in a wide variety of technologies and is not intended to be limiting. Any rejections made by examiners will be made under 35 U.S.C. 112, ¶1, with supporting rationale. Final rejections are appealable if applicant disagrees and follows the required procedures to appeal.

(14) *Comment:* Two comments indicated that if the amino acid sequence for a polypeptide whose utility has been identified is described, then the question of possession of a class of nucleotides encoding that polypeptide can be addressed as a relatively routine matter using the understanding of the genetic code, and that the endnote addressing this issue should be revised. *Response:* The suggestion of these comments has been incorporated in the Guidelines and will be reflected in the training materials. However, based upon *In re Bell*, 991 F.2d 781, 785, 26 USPQ2d 1529, 1532 (Fed. Cir. 1993) and *In re Baird*, 16 F.3d 380, 382, 29 USPQ2d 1550, 1552 (Fed. Cir. 1994), this does not mean that applicant was in possession of any particular species of the broad genus.

(15) *Comment:* One comment disagreed with an endnote which stated that a laundry list disclosure of moieties does not constitute a written description of every species in a genus. Specifically, the comment indicates that if the existence of a functional genus is adequately described in the specification, a laundry list of the species within that genus must satisfy the written description requirement. *Response:* The suggestion to revise the endnote will not be adopted. A lack of adequate written description problem arises if the knowledge and level of skill in the art would not permit one skilled in the art to immediately envisage the product claimed from the disclosure. This was aptly demonstrated in *In re Bell* and *In re Baird* where possession of a large genus did not put a person of ordinary skill in the art in possession of any particular species. *See also Purdue Pharma*, 230 F.3d at 1328, 56 USPQ2d at 1487 (because the original specification did not disclose the later claimed concentration ratio was a part of the invention, the inventors cannot argue that they are merely narrowing a broad invention).

(16) *Comment:* One comment suggested that in the majority of cases, a single species will support a generic claim, and that the Guidelines should emphasize this point. *Response:* The suggestion has been adopted to a limited degree. The Guidelines now indicate that a single species may, in some instances, provide an adequate written description of a generic claim when the description of the species would evidence to one of ordinary skill in the art that the invention includes the genus. Note, however, *Tronzo* v. *Biomet, Inc.*, 156 F.3d 1154, 47 USPQ2d 1829 (Fed. Cir. 1998), where the species in the parent application was held not to provide written description support for the genus in the child application.

(17) *Comment:* One comment asserted that the Guidelines should focus on the compliance of the claims, not the specification, with the written description requirement. *Response:* This suggestion will not be adopted. "The specification shall contain a written description of the invention." 35 U.S.C. 112. The claims are part of the specification. *Id.*, ¶ 2. If an adequate description is provided, it will suffice "whether located among the original claims or in the descriptive part of the specification." *In re Gardner*, 480 F.2d 879, 880, 178 USPQ 149 (CCPA 1973). The entire disclosure, including the specification, drawings, and claims, must be considered.

(18) *Comment:* One comment asserted that the Guidelines confuse "new matter," 35 U.S.C. 132, with the written description requirement, and that the same standard for written description should be applied to both original claims and new or amended claims. *Response:* The Guidelines indicate that for both original and amended claims, the inquiry is whether one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention at the time the application was filed.

(19) *Comment:* One comment suggested that the second paragraph of the section pertaining to determining what the claim as a whole covers should be deleted because it relates more to compliance with § 112, second paragraph, than with the written description requirement. *Response:* This suggestion will not be adopted. The claims must be construed and all issues as to the scope and meaning of the claim must be explored during the inquiry into whether the written description requirement has been met. The concept of treating the claim as a whole is applicable to all criteria for patentability.

(20) *Comment:* One comment suggested a different order for the general analysis for determining compliance with the written description requirement, starting with reading the claim, then the specification, and then determining whether the disclosure demonstrates possession by the applicant. *Response:* This suggestion will not be adopted. The claims must be construed as broadly as reasonable in light of the specification and the knowledge in the art. *See In re Morris*, 127 F.3d 1048, 1054, 44 USPQ2d 1023, 1027 (Fed. Cir. 1997). Then the disclosure must be evaluated to determine whether it adequately describes the claimed invention, i.e., whether it conveys to a person having ordinary skill in the art that the applicant had possession of what he or she now claims.

(21) *Comment:* Several comments suggested that the Guidelines are unclear with regard to how the examiner should treat the transitional phrase "consisting essentially of." The comments also suggested that the endnote that explains "consisting essentially of" does not make clear how the use of this intermediate transitional language affects the scope of the claim. Several comments stated that the USPTO does not have legal authority to treat claims reciting this language as open (equivalent to "comprising"). Another comment suggested that the phrase "clear indication in the specification" be replaced with "explicit or implicit indication." *Response:* The transitional phrase "consisting essentially of" "excludes

ingredients that would 'materially affect the basic and novel characteristics' of the claimed composition." *Atlas Powder Co.* v. *E.I. DuPont de Nemours & Co.*, 750 F.2d 1569, 1574, 224 USPQ 409, 412 (Fed. Cir. 1984). The basic and novel characteristics of the claimed invention are limited by the balance of the claim. *In re Janakirama-Rao*, 317 F.2d 951, 954, 137 USPQ 893, 896 (CCPA 1963). However, during prosecution claims must be read broadly, consistent with the specification. *In re Morris*, 127 F.3d 1048, 1054, 44 USPQ2d 1023, 1027 (Fed. Cir. 1997). Thus, *for purposes of searching for and applying prior art in a rejection under 35 U.S.C. 102 or 103*, if the specification or the claims do not define the "basic and novel" properties of the claimed subject matter (or if such properties are in dispute), the broadest reasonable interpretation consistent with the specification is that the basic and novel characteristics are merely the presence of the recited limitations. *See, e.g., Janakirama-Rao*, 317 F.2d at 954, 137 USPQ at 895–96. This does not indicate that the intermediate transitional language is never given weight. Applicants may amend the claims to avoid the rejections or seek to establish that the specification provides definitions of terms in the claims that define the basic and novel characteristics of the claimed invention which distinguish the claimed invention from the prior art. When an applicant contends that additional steps or materials in the prior art are excluded by the recitation of 'consisting essentially of,' applicant has the burden of showing that the introduction of additional steps or components would materially change the characteristics of applicant's invention. *In re De Lajarte*, 337 F.2d 870, 143 USPQ 256 (CCPA 1964). The language used in the Guidelines is consistent with *PPG Industries Inc.* v. *Guardian Industries Corp.*, 156 F.3d 1351, 1355, 48 USPQ2d 1351, 1355 (Fed. Cir. 1998) ("PPG could have defined the scope of the phrase 'consisting essentially of' for purposes of its patent by making clear in its specification what it regarded as constituting a material change in the basic and novel characteristics.").

(22) *Comment:* One comment stated that the written description should "disclose the invention," including why the invention works and how it was developed. *Response:* This suggestion has not been adopted. An inventor does not need to know how or why the invention works in order to obtain a patent. *Newman* v. *Quigg*, 877 F.2d 1575, 1581, 11 USPQ2d 1340, 1345 (Fed. Cir. 1989). To satisfy the enablement requirement of 35 U.S.C. 112, ¶1, an application must disclose the claimed invention in sufficient detail to enable a person of ordinary skill in the art to make and use the claimed invention. To satisfy the written description requirement of 35 U.S.C. 112, ¶ 1, the description must show that the applicant was in possession of the claimed invention at the time of filing. There is no statutory basis to require disclosure of why an invention works or how it was developed. "Patentability shall not be negatived by the manner in which the invention was made." 35 U.S.C. 103(a).

(23) *Comment:* One comment recommended that the phrases "emerging and unpredictable technologies" and "unpredictable art" be replaced with the phrase—inventions characterized by factors which are not reasonably predictable in terms of the ordinary skill in the art—. *Response:* The suggestion is adopted in part and the recommended phrase has been added as an alternative.

(24) *Comment:* One comment recommended that the phrase "conventional in the art" be replaced with—part of the knowledge of one of ordinary skill in the art—. *Response:* The suggestion is adopted in part and the recommended phrase has been added as an alternative. The standard of "conventional in the art" is supported by case law holding that a patent specification "need not teach, and preferably omits, what is well known in the art." *See Spectra-Physics, Inc.* v. *Coherent, Inc.*, 827 F.2d 1524, 1534, 3 USPQ2d 1737, 1743 (Fed. Cir. 1987); *Hybritech Inc.* v. *Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986). *See also Atmel Corp.* v. *Information Storage Devices, Inc.*, 198 F.3d 1374, 1382, 53 USPQ2d 1225, 1231 (Fed. Cir. 1999).

(25) *Comment:* One comment recommended that the Guidelines be amended to state that the appropriate skill level for determining possession of the claimed invention is that of a person of *ordinary* skill in the art. *Response:* The comment has not been adopted. The statutory language itself indicates that compliance with the requirements of 35 U.S.C. 112, ¶1, is judged from the standard of "any person skilled in the art." It is noted, however, that the phrases "one of skill in the art" and "one of ordinary skill in the art" appear to be synonymous. *See, e.g., Union Oil Co.* v. *Atlantic Richfield Co.*, 208 F.3d 989, 997, 54 USPQ2d 1227, 1232 (Fed. Cir. 2000) ("The written description requirement does not require the applicant 'to describe exactly the subject matter claimed, [instead] the description must clearly allow *persons of ordinary skill in the art* to recognize that [he or she] invented what is claimed.' Thus, § 112, ¶ 1, ensures that, as of the filing date, the inventor conveyed with reasonable clarity to *those of skill in the art* that he was in possession of the subject matter of the claims." (citations omitted, emphasis added)).

(26) *Comment:* One comment stated that an endnote misstates the relevant law in stating that, to show inherent written descriptive support for a claim limitation, the inherent disclosure must be such as would be recognized by a person of ordinary skill in the art. The comment recommended that the endnote be amended to delete the reference to recognition by persons of ordinary skill and to cite *Pingree* v. *Hull*, 518 F.2d 624, 186 USPQ 248 (CCPA 1975), rather than *In re Robertson*, 169 F.3d 743, 49 USPQ2d 1949 (Fed. Cir. 1999). *Response:* The comment has not been adopted. Federal Circuit precedent makes clear that an inherent disclosure must be recognized by those of ordinary skill in the art. *See, e.g., Hyatt* v. *Boone*, 146 F.3d 1348, 1354–55, 47 USPQ2d 1128, 1132 (Fed. Cir. 1998) ("[T]he purpose of the description requirement is 'to ensure that the inventor had possession, as of the filing date of the application relied on, of the specific subject matter later claimed by him.' * * * Thus, the written description must include all of the limitations of the interference count, or the applicant must show that any absent text is necessarily comprehended in the description provided *and would have been so understood* at the time the patent application was filed." (emphasis added)). *See also Reiffin* v. *Microsoft Corp.*, 214 F.3d 1342, 1346, 54 USPQ2d 1915, 1917 (Fed. Cir. 2000) (The "application considered as a whole must convey to one of ordinary skill in the art, either explicitly or inherently, that [the inventor] invented the subject matter claimed * * *. See * * * *Continental Can Co. USA* v. *Monsanto Co.*, 948 F.2d 1264, 1268, 20 USPQ2d 1746, 1749 (Fed. Cir. 1991) (descriptive matter may be inherently present in a specification if one skilled in the art would necessarily recognize such a disclosure)").

(27) *Comment:* Several comments pointed out an inconsistency in the **Federal Register** Notice re: the Revised Interim Written Description Guidelines. The inconsistency concerned the treatment of claims directed to an isolated DNA comprising SEQ ID NO:1 wherein SEQ ID NO:1 is an expressed sequence tag. The comments contrasted paragraphs 34 and 35 of the Response to

Public Comments with the statement in the text of the Guidelines that a genus must be supported by a representative number of species (as analyzed in Example 7 of the training materials). *Response:* The USPTO acknowledges that there was an inconsistency. The Office notes that a claim reciting a nucleic acid comprising SEQ ID NO:1 may be subject to a rejection for lack of an adequate written description where particular identifiable species within the scope of the claim lack an adequate written description. The training materials as amended exemplify an appropriate analysis.

(28) *Comment:* One comment stated that the USPTO should respond to the issue of whether the U.S. is meeting its TRIPs obligations. This comment noted that the USPTO did not address an earlier comment regarding the "Interim Guidelines for the Examination of Patent Applications under the 35 U.S.C. 112, ¶ 1, 'Written Description' Requirement," 63 FR 32,639, June 15, 1998, which questioned whether the written description requirement is truly different from the enablement requirement, and indicated that such a requirement may be contrary to the TRIPs provisions of the World Trade Organization (Article 27.1). Article 27.1 requires WTO Members to, *inter alia*, make patents available, with limited exceptions, for products and processes in all fields of technology so long as those products and processes are new, involve an inventive step, and are capable of industrial application. The comment further suggested a response. *Response:* TRIPs Article 27 does not address what must be included in a patent application to allow WTO Member officials to determine whether particular inventions meet the standards for patentability established in that Article. TRIPs Article 29, which is more relevant to this comment, states that Members "shall require" patent applicants to disclose their invention "in a manner sufficiently clear and complete for the invention to be carried out by a person skilled in the art." If the written description is not clear and complete, the applicant may not have been in possession of the invention. This may support both written description and enablement standards. In addition, Article 29 expressly authorizes Members to require patent applicants to disclose the best method the inventor knows at the time of filing an application for carrying out the invention.

(29) *Comment:* Two comments commended the USPTO for eliminating the Biotechnology Specific Examples in the Revised Interim Written Description Guidelines and providing separate training materials. One comment indicated a need to reconfirm the examples set forth in the Interim Written Description Guidelines published in 1998. *Response:* The current training materials reflect the manner in which the USPTO interprets the Written Description Guidelines.

(30) *Comment:* Several comments addressed specific concerns about the examiner training materials. *Response:* The comments received with respect to the training materials will be taken under advisement as the Office revises the training materials in view of the revisions to the Guidelines. The specific comments will not be addressed herein as they do not impact the language of the Guidelines.

Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1, "Written Description" Requirement

These "Written Description Guidelines" are intended to assist Office personnel in the examination of patent applications for compliance with the written description requirement of 35 U.S.C. 112, ¶ 1. This revision is based on the Office's current understanding of the law and public comments received in response to the USPTO's previous request for public comments on its Revised Interim Written Description Guidelines and is believed to be fully consistent with binding precedent of the U.S. Supreme Court, as well as the U.S. Court of Appeals for the Federal Circuit and its predecessor courts.

This revision does not constitute substantive rulemaking and hence does not have the force and effect of law. It is designed to assist Office personnel in analyzing claimed subject matter for compliance with substantive law. Rejections will be based upon the substantive law, and it is these rejections which are appealable. Consequently, any perceived failure by Office personnel to follow these Guidelines is neither appealable nor petitionable.

These Guidelines are intended to form part of the normal examination process. Thus, where Office personnel establish a *prima facie* case of lack of written description for a claim, a thorough review of the prior art and examination on the merits for compliance with the other statutory requirements, including those of 35 U.S.C. 101, 102, 103, and 112, is to be conducted prior to completing an Office action which includes a rejection for lack of written description. Office personnel are to rely on this revision of the Guidelines in the event of any inconsistent treatment of issues involving the written description requirement between these Guidelines and any earlier guidance provided from the Office.

I. General Principles Governing Compliance With the "Written Description" Requirement for Applications

The first paragraph of 35 U.S.C. 112 requires that the "specification shall contain a written description of the invention * * *." This requirement is separate and distinct from the enablement requirement.[1] The written description requirement has several policy objectives. "[T]he 'essential goal' of the description of the invention requirement is to clearly convey the information that an applicant has invented the subject matter which is claimed."[2] Another objective is to put the public in possession of what the applicant claims as the invention.[3] The written description requirement of the Patent Act promotes the progress of the useful arts by ensuring that patentees adequately describe their inventions in their patent specifications in exchange for the right to exclude others from practicing the invention for the duration of the patent's term.

To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention.[4] An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention.[5] Possession may be shown in a variety of ways including description of an actual reduction to practice,[6] or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas that show that the invention was complete,[7] or by describing distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention.[8] A question as to whether a specification provides an adequate written description may arise in the context of an original claim which is not described sufficiently, a new or amended claim wherein a claim limitation has been added or removed, or a claim to entitlement of an earlier priority date or effective filing date under 35 U.S.C. 119, 120, or 365(c).[9] Compliance with the written description requirement is a question of

fact which must be resolved on a case-by-case basis.[10]

A. Original Claims

There is a strong presumption that an adequate written description of the claimed invention is present when the application is filed.[11] However, the issue of a lack of adequate written description may arise even for an original claim when an aspect of the claimed invention has not been described with sufficient particularity such that one skilled in the art would recognize that the applicant had possession of the claimed invention.[12] The claimed invention as a whole may not be adequately described if the claims require an essential or critical feature which is not adequately described in the specification and which is not conventional in the art or known to one of ordinary skill in the art.[13] This problem may arise where an invention is described solely in terms of a method of its making coupled with its function and there is no described or art-recognized correlation or relationship between the structure of the invention and its function.[14] A lack of adequate written description issue also arises if the knowledge and level of skill in the art would not permit one skilled in the art to immediately envisage the product claimed from the disclosed process.[15]

B. New or Amended Claims

The proscription against the introduction of new matter in a patent application[16] serves to prevent an applicant from adding information that goes beyond the subject matter originally filed.[17] Thus, the written description requirement prevents an applicant from claiming subject matter that was not adequately described in the specification as filed. New or amended claims which introduce elements or limitations which are not supported by the as-filed disclosure violate the written description requirement.[18] While there is no *in haec verba* requirement, newly added claim limitations must be supported in the specification through express, implicit, or inherent disclosure. An amendment to correct an obvious error does not constitute new matter where one skilled in the art would not only recognize the existence of the error in the specification, but also recognize the appropriate correction.[19] Deposits made after the application filing date cannot be relied upon to support additions to or correction of information in the application as filed.[20]

Under certain circumstances, omission of a limitation can raise an issue regarding whether the inventor had possession of a broader, more generic invention.[21] A claim that omits an element which applicant describes as an essential or critical feature of the invention originally disclosed does not comply with the written description requirement.[22]

The fundamental factual inquiry is whether the specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, applicant was in possession of the invention as now claimed.[23]

II. Methodology for Determining Adequacy of Written Description

A. Read and Analyze the Specification for Compliance With 35 U.S.C. 112, ¶ 1

Office personnel should adhere to the following procedures when reviewing patent applications for compliance with the written description requirement of 35 U.S.C. 112, ¶ 1. The examiner has the initial burden, after a thorough reading and evaluation of the content of the application, of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims. There is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed;[24] however, with respect to newly added or amended claims, applicant should show support in the original disclosure for the new or amended claims.[25] Consequently, rejection of an original claim for lack of written description should be rare. The inquiry into whether the description requirement is met is a question of fact that must be determined on a case-by-case basis.[26]

1. For Each Claim, Determine What the Claim as a Whole Covers

Claim construction is an essential part of the examination process. Each claim must be separately analyzed and given its broadest reasonable interpretation in light of and consistent with the written description.[27] The entire claim must be considered, including the preamble language[28] and the transitional phrase.[29] The claim as a whole, including all limitations found in the preamble,[30] the transitional phrase, and the body of the claim, must be sufficiently supported to satisfy the written description requirement.[31]

The examiner should evaluate each claim to determine if sufficient structures, acts, or functions are recited to make clear the scope and meaning of the claim, including the weight to be given the preamble.[32] The absence of definitions or details for well-established terms or procedures should not be the basis of a rejection under 35 U.S.C. 112, ¶ 1, for lack of adequate written description. Limitations may not, however, be imported into the claims from the specification.

2. Review the Entire Application to Understand How Applicant Provides Support for the Claimed Invention Including Each Element and/or Step

Prior to determining whether the disclosure satisfies the written description requirement for the claimed subject matter, the examiner should review the claims and the entire specification, including the specific embodiments, figures, and sequence listings, to understand how applicant provides support for the various features of the claimed invention.[33] The analysis of whether the specification complies with the written description requirement calls for the examiner to compare the scope of the claim with the scope of the description to determine whether applicant has demonstrated possession of the claimed invention. Such a review is conducted from the standpoint of one of skill in the art at the time the application was filed[34] and should include a determination of the field of the invention and the level of skill and knowledge in the art. Generally, there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement. Information which is well known in the art need not be described in detail in the specification.[35]

3. Determine Whether There is Sufficient Written Description to Inform a Skilled Artisan That Applicant was in Possession of the Claimed Invention as a Whole at the Time the Application Was Filed

a. Original claims. Possession may be shown in many ways. For example, possession may be shown, *inter alia,* by describing an actual reduction to practice of the claimed invention. Possession may also be shown by a clear depiction of the invention in detailed drawings or in structural chemical formulas which permit a person skilled in the art to clearly recognize that applicant had possession of the claimed invention. An adequate written description of the invention may be shown by any description of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention.[36]

A specification may describe an actual reduction to practice by showing

that the inventor constructed an embodiment or performed a process that met all the limitations of the claim and determined that the invention would work for its intended purpose.[37] Description of an actual reduction to practice of a biological material may be shown by specifically describing a deposit made in accordance with the requirements of 37 CFR 1.801 *et seq.*[38]

An applicant may show possession of an invention by disclosure of drawings [39] or structural chemical formulas[40] that are sufficiently detailed to show that applicant was in possession of the claimed invention as a whole. The description need only describe in detail that which is new or not conventional.[41] This is equally true whether the claimed invention is directed to a product or a process.

An applicant may also show that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics [42] which provide evidence that applicant was in possession of the claimed invention,[43] *i.e.*, complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics.[44] What is conventional or well known to one of ordinary skill in the art need not be disclosed in detail.[45] If a skilled artisan would have understood the inventor to be in possession of the claimed invention at the time of filing, even if every nuance of the claims is not explicitly described in the specification, then the adequate description requirement is met.[46]

(1) For each claim drawn to a single embodiment or species: [47]

(a) Determine whether the application describes an actual reduction to practice of the claimed invention.

(b) If the application does not describe an actual reduction to practice, determine whether the invention is complete as evidenced by a reduction to drawings or structural chemical formulas that are sufficiently detailed to show that applicant was in possession of the claimed invention as a whole.

(c) If the application does not describe an actual reduction to practice or reduction to drawings or structural chemical formula as discussed above, determine whether the invention has been set forth in terms of distinguishing identifying characteristics as evidenced by other descriptions of the invention that are sufficiently detailed to show that applicant was in possession of the claimed invention.

(i) Determine whether the application as filed describes the complete structure (or acts of a process) of the claimed invention as a whole. The complete structure of a species or embodiment typically satisfies the requirement that the description be set forth "in such full, clear, concise, and exact terms" to show possession of the claimed invention.[48] If a complete structure is disclosed, the written description requirement is satisfied for that species or embodiment, and a rejection under 35 U.S.C. 112, ¶ 1, for lack of written description must not be made.

(ii) If the application as filed does not disclose the complete structure (or acts of a process) of the claimed invention as a whole, determine whether the specification discloses other relevant identifying characteristics sufficient to describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize applicant was in possession of the claimed invention.[49]

Whether the specification shows that applicant was in possession of the claimed invention is not a single, simple determination, but rather is a factual determination reached by considering a number of factors. Factors to be considered in determining whether there is sufficient evidence of possession include the level of skill and knowledge in the art, partial structure, physical and/or chemical properties, functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and the method of making the claimed invention. Disclosure of any combination of such identifying characteristics that distinguish the claimed invention from other materials and would lead one of skill in the art to the conclusion that the applicant was in possession of the claimed species is sufficient.[50] Patents and printed publications in the art should be relied upon to determine whether an art is mature and what the level of knowledge and skill is in the art. In most technologies which are mature, and wherein the knowledge and level of skill in the art is high, a written description question should not be raised for original claims even if the specification discloses only a method of making the invention and the function of the invention.[51] In contrast, for inventions in emerging and unpredictable technologies, or for inventions characterized by factors not reasonably predictable which are known to one of ordinary skill in the art, more evidence is required to show possession. For example, disclosure of only a method of making the invention and the function may not be sufficient to support a product claim other than a product-by-process claim.[52] Furthermore, disclosure of a partial structure without additional characterization of the product may not be sufficient to evidence possession of the claimed invention.[53]

Any claim to a species that does not meet the test described under at least one of (a), (b), or (c) must be rejected as lacking adequate written description under 35 U.S.C. 112, ¶ 1.

(2) For each claim drawn to a genus:

The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice (see (1)(a), above), reduction to drawings (see (1)(b), above), or by disclosure of relevant, identifying characteristics, *i.e.*, structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus (see (1)(c), above).[54]

A "representative number of species" means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. On the other hand, there may be situations where one species adequately supports a genus.[55] What constitutes a "representative number" is an inverse function of the skill and knowledge in the art. Satisfactory disclosure of a "representative number" depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. For inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species *cannot* be achieved by disclosing only one species within the genus.[56] Description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces.[57] If a representative number of adequately described species are not disclosed for a genus, the claim to that genus must be rejected as lacking adequate written description under 35 U.S.C. 112, ¶ 1.

b. New claims, amended claims, or claims asserting entitlement to the benefit of an earlier priority date or filing date under 35 U.S.C. 119, 120, or

365(c). The examiner has the initial burden of presenting evidence or reasoning to explain why persons skilled in the art would not recognize in the original disclosure a description of the invention defined by the claims.[58] However, when filing an amendment an applicant should show support in the original disclosure for new or amended claims.[59] To comply with the written description requirement of 35 U.S.C. 112, ¶ 1, or to be entitled to an earlier priority date or filing date under 35 U.S.C. 119, 120, or 365(c), each claim limitation must be expressly,[60] implicitly,[61] or inherently[62] supported in the originally filed disclosure.[63] Furthermore, each claim must include all elements which applicant has described as essential.[64]

If the originally filed disclosure does not provide support for each claim limitation, or if an element which applicant describes as essential or critical is not claimed, a new or amended claim must be rejected under 35 U.S.C. 112, ¶ 1, as lacking adequate written description, or in the case of a claim for priority under 35 U.S.C. 119, 120, or 365(c), the claim for priority must be denied.

III. Complete Patentability Determination Under All Statutory Requirements and Clearly Communicate Findings, Conclusions, and Their Bases

The above only describes how to determine whether the written description requirement of 35 U.S.C. 112, ¶ 1, is satisfied. Regardless of the outcome of that determination, Office personnel must complete the patentability determination under all the relevant statutory provisions of title 35 of the U.S. Code.

Once Office personnel have concluded analysis of the claimed invention under all the statutory provisions, including 35 U.S.C. 101, 112, 102, and 103, they should review all the proposed rejections and their bases to confirm their correctness. Only then should any rejection be imposed in an Office action. The Office action should clearly communicate the findings, conclusions, and reasons which support them. When possible, the Office action should offer helpful suggestions on how to overcome rejections.

A. For Each Claim Lacking Written Description Support, Reject the Claim Under Section 112, ¶1, for Lack of Adequate Written Description

A description as filed is presumed to be adequate, unless or until sufficient evidence or reasoning to the contrary has been presented by the examiner to rebut the presumption.[65] The examiner, therefore, must have a reasonable basis to challenge the adequacy of the written description. The examiner has the initial burden of presenting by a preponderance of evidence why a person skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims.[66] In rejecting a claim, the examiner must set forth express findings of fact regarding the above analysis which support the lack of written description conclusion. These findings should:

(1) Identify the claim limitation at issue; and

(2) Establish a *prima facie* case by providing reasons why a person skilled in the art at the time the application was filed would not have recognized that the inventor was in possession of the invention as claimed in view of the disclosure of the application as filed. A general allegation of "unpredictability in the art" is not a sufficient reason to support a rejection for lack of adequate written description.

When appropriate, suggest amendments to the claims which can be supported by the application's written description, being mindful of the prohibition against the addition of new matter in the claims or description.[67]

B. Upon Reply by Applicant, Again Determine the Patentability of the Claimed Invention, Including Whether the Written Description Requirement Is Satisfied by Reperforming the Analysis Described Above in View of the Whole Record

Upon reply by applicant, before repeating any rejection under 35 U.S.C. 112, ¶ 1, for lack of written description, review the basis for the rejection in view of the record as a whole, including amendments, arguments, and any evidence submitted by applicant. If the whole record now demonstrates that the written description requirement is satisfied, do *not* repeat the rejection in the next Office action. If the record still does not demonstrate that the written description is adequate to support the claim(s), repeat the rejection under 35 U.S.C. 112, ¶ 1, fully respond to applicant's rebuttal arguments, and properly treat any further showings submitted by applicant in the reply. When a rejection is maintained, any affidavits relevant to the 112, ¶ 1, written description requirement,[68] must be thoroughly analyzed and discussed in the next Office action.

Dated: December 29, 2000.

Q. Todd Dickinson,

Under Secretary of Commerce for Intellectual Property and Director of the United States Patent and Trademark Office.

Endnotes

[1] *See, e.g., Vas-Cath, Inc.* v. *Mahurkar*, 935 F.2d 1555, 1560, 19 USPQ2d 1111, 1114 (Fed. Cir. 1991).

[2] *In re Barker*, 559 F.2d 588, 592 n.4, 194 USPQ 470, 473 n.4 (CCPA 1977).

[3] *See Regents of the University of California* v. *Eli Lilly*, 119 F.3d 1559, 1566, 43 USPQ2d 1398, 1404 (Fed. Cir. 1997), *cert. denied*, 523 U.S. 1089 (1998).

[4] *See, e.g., Vas-Cath, Inc.* v. *Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116. Much of the written description case law addresses whether the specification as originally filed supports claims not originally in the application. The issue raised in the cases is most often phrased as whether the original application provides "adequate support" for the claims at issue or whether the material added to the specification incorporates "new matter" in violation of 35 U.S.C. 132. The "written description" question similarly arises in the interference context, where the issue is whether the specification of one party to the interference can support the newly added claims corresponding to the count at issue, *i.e.*, whether that party can "make the claim" corresponding to the interference count. *See, e.g., Martin* v. *Mayer*, 823 F.2d 500, 503, 3 USPQ2d 1333, 1335 (Fed. Cir. 1987).

In addition, early opinions suggest the Patent and Trademark Office was unwilling to find written descriptive support when the only description was found in the claims; however, this viewpoint was rejected. *See In re Koller*, 613 F.2d 819, 204 USPQ 702 (CCPA 1980) (original claims constitute their own description); *accord In re Gardner*, 475 F.2d 1389, 177 USPQ 396 (CCPA 1973); *accord In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976) (accord). It is now well accepted that a satisfactory description may be in the claims or any other portion of the originally filed specification. These early opinions did not address the quality or specificity of particularity that was required in the description, *i.e.*, how much description is enough.

[5] *Lockwood* v. *American Airlines, Inc.*, 107 F.3d 1565, 1572, 41 USPQ2d 1961, 1966 (Fed. Cir. 1997).

[6] An application specification may show actual reduction to practice by describing testing of the claimed invention or, in the case of biological materials, by specifically describing a deposit made in accordance with 37 CFR 1.801 *et seq. See also Deposit of Biological Materials for Patent Purposes, Final Rule*, 54 FR 34,864 (August 22, 1989) ("The requirement for a specific identification is consistent with the description requirement of the first paragraph of 35 U.S.C. 112, and to provide an antecedent basis for the biological material which either has been or will be deposited before the patent is granted." *Id.* at 34,876. "The description must be sufficient to permit verification that the deposited biological material is in fact that disclosed. Once the

patent issues, the description must be sufficient to aid in the resolution of questions of infringement." *Id.* at 34,880.). Such a deposit is not a substitute for a written description of the claimed invention. The written description of the deposited material needs to be as complete as possible because the examination for patentability proceeds solely on the basis of the written description. *See, e.g., In re Lundak*, 773 F.2d 1216, 227 USPQ 90 (Fed. Cir. 1985). *See also* 54 FR at 34,880 ("As a general rule, the more information that is provided about a particular deposited biological material, the better the examiner will be able to compare the identity and characteristics of the deposited biological material with the prior art.").

[7] *Pfaff* v. *Wells Electronics, Inc.*, 525 U.S. 55, 68, 119 S.Ct. 304, 312, 48 USPQ2d 1641, 1647 (1998); *Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

[8] *See Amgen, Inc.* v. *Chugai Pharmaceutical*, 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991) (one must define a compound by "whatever characteristics sufficiently distinguish it").

[9] A description requirement issue can arise for original claims (*see, e.g., Eli Lilly*, 119 F.3d 1559, 43 USPQ2d 1398) as well as new or amended claims. Most typically, the issue will arise in the context of determining whether new or amended claims are supported by the description of the invention in the application as filed (*see, e.g., In re Wright*, 866 F.2d 422, 9 USPQ2d 1649 (Fed. Cir. 1989)), whether a claimed invention is entitled to the benefit of an earlier priority date or effective filing date under 35 U.S.C. 119, 120, or 365(c) (*see, e.g., Tronzo* v. *Biomet, Inc.*, 156 F.3d 1154, 47 USPQ2d 1829 (Fed. Cir. 1998); *Fiers* v. *Revel*, 984 F.2d 1164, 25 USPQ2d 1601 (Fed. Cir. 1993); *In re Ziegler*, 992 F.2d 1197, 1200, 26 USPQ2d 1600, 1603 (Fed. Cir. 1993)), or whether a specification provides support for a claim corresponding to a count in an interference (*see, e.g., Fields* v. *Conover*, 443 F.2d 1386, 170 USPQ 276 (CCPA 1971)).

[10] *Vas-Cath, Inc.* v. *Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116 (Fed. Cir. 1991).

[11] *In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976) ("we are of the opinion that the PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims").

[12] See endnote 4.

[13] For example, consider the claim "A gene comprising SEQ ID NO:1." A determination of what the claim as a whole covers may result in a conclusion that specific structures such as a promoter, a coding region, or other elements are included. Although all genes encompassed by this claim share the characteristic of comprising SEQ ID NO:1, there may be insufficient description of those specific structures (*e.g.*, promoters, enhancers, coding regions, and other regulatory elements) which are also included.

[14] A biomolecule sequence described only by a functional characteristic, without any known or disclosed correlation between that function and the structure of the sequence, normally is not a sufficient identifying characteristic for written description purposes, even when accompanied by a method of obtaining the claimed sequence. For example, even though a genetic code table would correlate a known amino acid sequence with a genus of coding nucleic acids, the same table cannot predict the native, naturally occurring nucleic acid sequence of a naturally occurring mRNA or its corresponding cDNA. *Cf. In re Bell*, 991 F.2d 781, 26 USPQ2d 1529 (Fed. Cir. 1993), and *In re Deuel*, 51 F.3d 1552, 34 USPQ2d 1210 (Fed. Cir. 1995) (holding that a process could not render the product of that process obvious under 35 U.S.C. 103). The Federal Circuit has pointed out that under United States law, a description that does not render a claimed invention obvious cannot sufficiently describe the invention for the purposes of the written description requirement of 35 U.S.C. 112. *Eli Lilly*, 119 F.3d at 1567, 43 USPQ2d at 1405.

Compare Fonar Corp. v. *General Electric Co.*, 107 F.3d 1543, 1549, 41 USPQ2d 1801, 1805 (Fed. Cir. 1997) ("As a general rule, where software constitutes part of a best mode of carrying out an invention, description of such a best mode is satisfied by a disclosure of the functions of the software. This is because, normally, writing code for such software is within the skill of the art, not requiring undue experimentation, once its functions have been disclosed. * * * Thus, flow charts or source code listings are not a requirement for adequately disclosing the functions of software.").

[15] *See, e.g., Fujikawa* v. *Wattanasin*, 93 F.3d 1559, 1571, 39 USPQ2d 1895, 1905 (Fed. Cir. 1996) (a "laundry list" disclosure of every possible moiety does not constitute a written description of every species in a genus because it would not "reasonably lead" those skilled in the art to any particular species); *In re Ruschig*, 379 F.2d 990, 995, 154 USPQ 118, 123 (CCPA 1967) ("*If* n-propylamine had been used in making the compound instead of n-butylamine, the compound of claim 13 would have resulted. Appellants submit to us, as they did to the board, an imaginary specific example patterned on specific example 6 by which the above butyl compound is made so that we can see what a simple change would have resulted in a specific supporting disclosure being present in the present specification. The trouble is that there is no such disclosure, easy though it is to imagine it.") (emphasis in original); *Purdue Pharma L.P.* v. *Faulding Inc.*, 230 F.3d 1320, 1328, 56 USPQ2d 1481, 1487 (Fed. Cir. 2000) ("the specification does not clearly disclose to the skilled artisan that the inventors * * * considered the [] ratio to be part of their invention * * *. There is therefore no force to Purdue's argument that the written description requirement was satisfied because the disclosure revealed a broad invention from which the [later-filed] claims carved out a patentable portion").

[16] 35 U.S.C. §§ 132 and 251. *See also In re Rasmussen*, 650 F.2d 1212, 1214, 211 USPQ 323, 326 (CCPA 1981). *See* Manual of Patent Examining Procedure (MPEP) §§ 2163.06–2163.07 (7th Ed., Rev. 1, Feb. 2000) for a more detailed discussion of the written description requirement and its relationship to new matter.

[17] The claims as filed in the original specification are part of the disclosure and, therefore, if an application as originally filed contains a claim disclosing material not found in the remainder of the specification, the applicant may amend the specification to include the claimed subject matter. *In re Benno*, 768 F.2d 1340, 226 USPQ 683 (Fed. Cir. 1985).

[18] *See, e.g., In re Lukach*, 442 F.2d 967, 169 USPQ 795 (CCPA 1971) (subgenus range was not supported by generic disclosure and specific example within the subgenus range); *In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972) (a subgenus is not necessarily described by a genus encompassing it and a species upon which it reads).

[19] *In re Oda*, 443 F.2d 1200, 170 USPQ 260 (CCPA 1971). With respect to the correction of sequencing errors in applications disclosing nucleic acid and/or amino acid sequences, it is well known that sequencing errors are a common problem in molecular biology. *See, e.g.*, Peter Richterich, *Estimation of Errors in 'Raw' DNA Sequences: A Validation Study*, 8 Genome Research 251–59 (1998). If an application as filed includes sequence information and references a deposit of the sequenced material made in accordance with the requirements of 37 CFR § 1.801 *et seq.*, amendment may be permissible.

[20] Corrections of minor errors in the sequence may be possible based on the argument that one of skill in the art would have resequenced the deposited material and would have immediately recognized the minor error. Deposits made after the filing date can only be relied upon to provide support for the correction of sequence information if applicant submits a statement in compliance with 37 CFR § 1.804 stating that the biological material which is deposited is a biological material specifically defined in the application as filed.

[21] *See, e.g., Gentry Gallery, Inc.* v. *Berkline Corp.*, 134 F.3d 1473, 45 USPQ2d 1498 (Fed. Cir. 1998) (claims to a sectional sofa comprising, *inter alia*, a console and a control means were held invalid for failing to satisfy the written description requirement where the claims were broadened by removing the location of the control means.); *Johnson Worldwide Associates* v. *Zebco Corp.*, 175 F.3d 985, 993, 50 USPQ2d 1607, 1613 (Fed. Cir. 1999) (In *Gentry Gallery*, the "court's determination that the patent disclosure did not support a broad meaning for the disputed claim terms was premised on clear statements in the written description that described the location of a claim element—the 'control means'—as 'the only possible location' and that variations were 'outside the stated purpose of the invention.' *Gentry Gallery*, 134 F.3d at 1479, 45 USPQ2d at 1503. *Gentry Gallery*, then, considers the situation where the patent's disclosure makes crystal clear that a particular (*i.e.*, narrow) understanding of a claim term is an 'essential element of [the inventor's] invention.'"); *Tronzo* v. *Biomet*, 156 F.3d at 1158–59, 47 USPQ2d at 1833 (Fed. Cir. 1998) (claims to generic cup shape were not entitled to filing date of parent application which disclosed "conical cup" in view of the disclosure of the

parent application stating the advantages and importance of the conical shape.).

[22] *See Gentry Gallery*, 134 F.3d at 1480, 45 USPQ2d at 1503; *In re Sus*, 306 F.2d 494, 504, 134 USPQ 301, 309 (CCPA 1962) ("[O]ne skilled in this art would not be taught by the written description of the invention in the specification that any 'aryl or substituted aryl radical' would be suitable for the purposes of the invention but rather that only *certain aryl radicals* and certain specifically substituted aryl radicals [*i.e.*, aryl azides] would be suitable for such purposes.") (emphasis in original). A claim which omits matter disclosed to be essential to the invention as described in the specification or in other statements of record may also be subject to rejection under 35 U.S.C. 112, ¶ 1, as not enabling, or under 35 U.S.C. 112, ¶ 2. *See In re Mayhew*, 527 F.2d 1229, 188 USPQ 356 (CCPA 1976); *In re Venezia*, 530 F.2d 956, 189 USPQ 149 (CCPA 1976); and *In re Collier*, 397 F.2d 1003, 158 USPQ 266 (CCPA 1968). *See also* MPEP § 2172.01.

[23] *See, e.g., Vas-Cath, Inc.*, 935 F.2d at 1563–64, 19 USPQ2d at 1117.

[24] *Wertheim*, 541 F.2d at 262, 191 USPQ at 96.

[25] *See* MPEP §§ 714.02 and 2163.06 ("Applicant should * * * specifically point out the support for any amendments made to the disclosure."); and MPEP § 2163.04 ("If applicant amends the claims and points out where and/or how the originally filed disclosure supports the amendment(s), and the examiner finds that the disclosure does not reasonably convey that the inventor had possession of the subject matter of the amendment at the time of the filing of the application, the examiner has the initial burden of presenting evidence or reasoning to explain why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims.").

[26] *See In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972) ("Precisely how close [to the claimed invention] the description must come to comply with § 112 must be left to case-by-case development."); *In re Wertheim*, 541 F.2d at 262, 191 USPQ at 96 (inquiry is primarily factual and depends on the nature of the invention and the amount of knowledge imparted to those skilled in the art by the disclosure).

[27] *See, e.g., In re Morris*, 127 F.3d 1048, 1053–54, 44 USPQ2d 1023, 1027 (Fed. Cir. 1997).

[28] "Preamble language" is that language in a claim appearing before the transitional phase, *e.g.*, before "comprising," "consisting essentially of," or "consisting of."

[29] The transitional term "comprising" (and other comparable terms, *e.g.*, "containing," "including," and "having") is "open-ended—it covers the expressly recited subject matter, alone or in combination with unrecited subject matter. *See, e.g., Genentech, Inc.* v. *Chiron Corp.*, 112 F.3d 495, 501, 42 USPQ2d 1608, 1613 (Fed. Cir. 1997) ("'Comprising' is a term of art used in claim language which means that the named elements are essential, but other elements may be added and still form a construct within the scope of the claim."); *Ex parte Davis*, 80 USPQ 448, 450 (Bd. App. 1948) ("comprising" leaves the "claim open for the inclusion of unspecified ingredients even in major amounts"). "By using the term 'consisting essentially of,' the drafter signals that the invention necessarily includes the listed ingredients and is open to unlisted ingredients that do not materially affect the basic and novel properties of the invention. A 'consisting essentially of' claim occupies a middle ground between closed claims that are written in a 'consisting of' format and fully open claims that are drafted in a 'comprising' format." *PPG Industries* v. *Guardian Industries*, 156 F.3d 1351, 1354, 48 USPQ2d 1351, 1353–54 (Fed. Cir. 1998). For the purposes of searching for and applying prior art under 35 U.S.C. 102 and 103, absent a clear indication in the specification or claims of what the basic and novel characteristics actually are, 'consisting essentially of' will be construed as equivalent to "comprising." *See, e.g., PPG*, 156 F.3d at 1355, 48 USPQ2d at 1355 ("PPG could have defined the scope of the phrase "consisting essentially of" for purposes of its patent by making clear in its specification what it regarded as constituting a material change in the basic and novel characteristics of the invention."). *See also In re Janakirama-Rao*, 317 F.2d 951, 954, 137 USPQ 893, 895–96 (CCPA 1963). If an applicant contends that additional steps or materials in the prior art are excluded by the recitation of "consisting essentially of," applicant has the burden of showing that the introduction of additional steps or components would materially change the characteristics of applicant's invention. *In re De Lajarte*, 337 F.2d 870, 143 USPQ 256 (CCPA 1964).

[30] *See Pac-Tec Inc.* v. *Amerace Corp.*, 903 F.2d 796, 801, 14 USPQ2d 1871, 1876 (Fed. Cir. 1990) (determining that preamble language that constitutes a structural limitation is actually part of the claimed invention).

[31] An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations. *Lockwood*, 107 F.3d at 1572, 41 USPQ2d at 1966.

[32] *See, e.g., Bell Communications Research, Inc.* v. *Vitalink Communications Corp.*, 55 F.3d 615, 620, 34 USPQ2d 1816, 1820 (Fed. Cir. 1995) ("[A] claim preamble has the import that the claim as a whole suggests for it."); *Corning Glass Works* v. *Sumitomo Elec. U.S.A., Inc.*, 868 F.2d 1251, 1257, 9 USPQ2d 1962, 1966 (Fed. Cir. 1989) (The determination of whether preamble recitations are structural limitations can be resolved only on review of the entirety of the application "to gain an understanding of what the inventors actually invented and intended to encompass by the claim.").

[33] An element may be critical where those of skill in the art would require it to determine that applicant was in possession of the invention. *Compare Rasmussen*, 650 F.2d at 1215, 211 USPQ at 327 ("one skilled in the art who read Rasmussen's specification would understand that it is unimportant *how* the layers are adhered, so long as they are adhered") (emphasis in original), *with Amgen, Inc.* v. *Chugai Pharmaceutical Co., Ltd.*, 927 F.2d 1200, 1206, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991) ("it is well established in our law that conception of a chemical compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it").

[34] *See, e.g., Wang Labs.* v. *Toshiba Corp.*, 993 F.2d 858, 865, 26 USPQ2d 1767, 1774 (Fed. Cir. 1993).

[35] *See, e.g., Hybritech, Inc.* v. *Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379–80, 231 USPQ 81, 90 (Fed. Cir. 1986).

[36] *See, e.g., Purdue Pharma L.P.* v. *Faulding Inc.*, 230 F.3d 1320, ___, 56 USPQ2d 1481, 1483 (Fed. Cir. 2000) (the written description "inquiry is a factual one and must be assessed on a case-by-case basis"); *see also Pfaff* v. *Wells Electronics, Inc.*, 55 U.S. at 66, 119 S.Ct. at 311, 48 USPQ2d at 1646 ("The word 'invention' must refer to a concept that is complete, rather than merely one that is 'substantially complete.' It is true that reduction to practice ordinarily provides the best evidence that an invention is complete. But just because reduction to practice is sufficient evidence of completion, it does not follow that proof of reduction to practice is necessary in every case. Indeed, both the facts of the *Telephone Cases* and the facts of this case demonstrate that one can prove that an invention is complete and ready for patenting before it has actually been reduced to practice.").

[37] *Cooper* v. *Goldfarb*, 154 F.3d 1321, 1327, 47 USPQ2d 1896, 1901 (Fed. Cir. 1998). *See also UMC Elecs. Co.* v. *United States*, 816 F.2d 647, 652, 2 USPQ2d 1465, 1468 (Fed. Cir. 1987) ("[T]here cannot be a reduction to practice of the invention * * * without a physical embodiment which includes all limitations of the claim."); *Estee Lauder Inc.* v. *L'Oreal, S.A.*, 129 F.3d 588, 593, 44 USPQ2d 1610, 1614 (Fed. Cir. 1997) ("[A] reduction to practice does not occur until the inventor has determined that the invention will work for its intended purpose."); *Mahurkar* v. *C.R. Bard, Inc.*, 79 F.3d 1572, 1578, 38 USPQ2d 1288, 1291 (Fed. Cir. 1996) (determining that the invention will work for its intended purpose may require testing depending on the character of the invention and the problem it solves).

[38] 37 CFR 1.804, 1.809. *See also* endnote 6.

[39] *See, e.g., Vas-Cath*, 935 F.2d at 1565, 19 USPQ2d at 1118 ("drawings alone may provide a 'written description' of an invention as required by § 112"); *In re Wolfensperger*, 302 F.2d 950, 133 USPQ 537 (CCPA 1962) (the drawings of applicant's specification provided sufficient written descriptive support for the claim limitation at issue); *Autogiro Co. of America* v. *United States*, 384 F.2d 391, 398, 155 USPQ 697, 703 (Ct. Cl. 1967) ("In those instances where a visual representation can flesh out words, drawings may be used in the same manner and with the same limitations as the specification.").

[40] *See, e.g., Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406 ("In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus.").

[41] *See Hybritech* v. *Monoclonal Antibodies*, 802 F.2d at 1384, 231 USPQ at 94; *Fonar Corp.* v. *General Electric Co.*, 107 F.3d at 1549, 41 USPQ2d at 1805 (source code description not required).

[42] For example, the presence of a restriction enzyme map of a gene may be relevant to a statement that the gene has been isolated. One skilled in the art may be able to determine when the gene disclosed is the same as or different from a gene isolated by another by comparing the restriction enzyme map. In contrast, evidence that the gene could be digested with a nuclease would not normally represent a relevant characteristic since any gene would be digested with a nuclease. Similarly, isolation of an mRNA and its expression to produce the protein of interest is strong evidence of possession of an mRNA for the protein.

For some biomolecules, examples of identifying characteristics include a sequence, structure, binding affinity, binding specificity, molecular weight, and length. Although structural formulas provide a convenient method of demonstrating possession of specific molecules, other identifying characteristics or combinations of characteristics may demonstrate the requisite possession. For example, unique cleavage by particular enzymes, isoelectric points of fragments, detailed restriction enzyme maps, a comparison of enzymatic activities, or antibody cross-reactivity may be sufficient to show possession of the claimed invention to one of skill in the art. *See Lockwood*, 107 F.3d at 1572, 41 USPQ2d at 1966 ("written description" requirement may be satisfied by using "such descriptive means as words, structures, figures, diagrams, formulas, etc., that fully set forth the claimed invention").

[43] A definition by function alone "does not suffice" to sufficiently describe a coding sequence "because it is only an indication of what the gene does, rather than what it is." *Eli Lilly*, 119 F.3 at 1568, 43 USPQ2d at 1406. *See also Fiers*, 984 F.2d at 1169–71, 25 USPQ2d at 1605–06 (discussing *Amgen Inc.* v. *Chugai Pharmaceutical Co.*, 927 F.2d 1200, 18 USPQ2d 1016 (Fed. Cir. 1991)).

[44] If a claim limitation invokes 35 U.S.C. 112, ¶ 6, it must be interpreted to cover the corresponding structure, materials, or acts in the specification and "equivalents thereof." *See* 35 U.S.C. 112, ¶ 6. *See also B. Braun Medical, Inc.* v. *Abbott Lab.*, 124 F.3d 1419, 1424, 43 USPQ2d 1896, 1899 (Fed. Cir. 1997). In considering whether there is 35 U.S.C. 112, ¶ 1, support for a means- (or step) plus-function claim limitation, the examiner must consider not only the original disclosure contained in the summary and detailed description of the invention portions of the specification, but also the original claims, abstract, and drawings. A means- (or step-) plus-function claim limitation is adequately described under 35 U.S.C. 112, ¶ 1, if: (1) The written description adequately links or associates adequately described particular structure, material, or acts to the function recited in a means- (or step-) plus-function claim limitation; or (2) it is clear based on the facts of the application that one skilled in the art would have known what structure, material, or acts perform the function recited in a means- (or step-) plus-function limitation. Note also: A rejection under 35 U.S.C. 112, ¶ 2, "cannot stand where there is adequate description in the specification to satisfy 35 U.S.C. 112, first paragraph, regarding means-plus-function recitations that are not, per se, challenged for being unclear." *In re Noll*, 545 F.2d 141, 149, 191 USPQ 721, 727 (CCPA 1976). *See Supplemental Examination Guidelines for Determining the Applicability of 35 U.S.C. 112, ¶ 6*, 65 FR 38510, June 21, 2000.

[45] *See Hybritech Inc.* v. *Monoclonal Antibodies, Inc.*, 802 F.2d at 1384, 231 USPQ at 94.

[46] *See, e.g., Vas-Cath*, 935 F.2d at 1563, 19 USPQ2d at 1116; *Martin* v. *Johnson*, 454 F.2d 746, 751, 172 USPQ 391, 395 (CCPA 1972) (stating "the description need not be in *ipsis verbis* [*i.e.*, "in the same words"] to be sufficient").

[47] A claim which is limited to a single disclosed embodiment or species is analyzed as a claim drawn to a single embodiment or species, whereas a claim which encompasses two or more embodiments or species within the scope of the claim is analyzed as a claim drawn to a genus. *See also* MPEP § 806.04(e).

[48] 35 U.S.C. 112, ¶ 1. *Cf. Fields* v. *Conover*, 443 F.2d 1386, 1392, 170 USPQ 276, 280 (CCPA 1971) (finding a lack of written description because the specification lacked the "full, clear, concise, and exact written description" which is necessary to support the claimed invention).

[49] For example, if the art has established a strong correlation between structure and function, one skilled in the art would be able to predict with a reasonable degree of confidence the structure of the claimed invention from a recitation of its function. Thus, the written description requirement may be satisfied through disclosure of function and minimal structure when there is a well-established correlation between structure and function. In contrast, without such a correlation, the capability to recognize or understand the structure from the mere recitation of function and minimal structure is highly unlikely. In this latter case, disclosure of function alone is little more than a wish for possession; it does not satisfy the written description requirement. *See Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406 (written description requirement not satisfied by merely providing "a result that one might achieve if one made that invention"); *In re Wilder*, 736 F.2d 1516, 1521, 222 USPQ 369, 372–73 (Fed. Cir. 1984) (affirming a rejection for lack of written description because the specification does "little more than outline goals appellants hope the claimed invention achieves and the problems the invention will hopefully ameliorate"). *Compare Fonar*, 107 F.3d at 1549, 41 USPQ2d at 1805 (disclosure of software function adequate in that art).

[50] *See Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

[51] *See, e.g., In re Hayes Microcomputer Products, Inc. Patent Litigation*, 982 F.2d 1527, 1534–35, 25 USPQ2d 1241, 1246 (Fed. Cir. 1992) ("One skilled in the art would know how to program a microprocessor to perform the necessary steps described in the specification. Thus, an inventor is not required to describe every detail of his invention. An applicant's disclosure obligation varies according to the art to which the invention pertains. Disclosing a microprocessor capable of performing certain functions is sufficient to satisfy the requirement of section 112, first paragraph, when one skilled in the relevant art would understand what is intended and know how to carry it out.")

[52] *See, e.g., Fiers* v. *Revel*, 984 F.2d at 1169, 25 USPQ2d at 1605; *Amgen.*, 927 F.2d at 1206, 18 USPQ2d at1021. Where the process has actually been used to produce the product, the written description requirement for a product-by-process claim is clearly satisfied; however, the requirement may not be satisfied where it is not clear that the acts set forth in the specification can be performed, or that the product is produced by that process.

[53] *See, e.g., Amgen*, 927 F.2d at1206, 18 USPQ2d at 1021 ("A gene is a chemical compound, albeit a complex one, and it is well established in our law that conception of a chemical compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it. Conception does not occur unless one has a mental picture of the structure of the chemical, or is able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics sufficiently distinguish it. It is not sufficient to define it solely by its principal biological property, *e.g.*, encoding human erythropoietin, because an alleged conception having no more specificity than that is simply a wish to know the identity of any material with that biological property. We hold that when an inventor is unable to envision the detailed constitution of a gene so as to distinguish it from other materials, as well as a method for obtaining it, conception has not been achieved until reduction to practice has occurred, *i.e.*, until after the gene has been isolated.") (citations omitted). In such instances the alleged conception fails not merely because the field is unpredictable or because of the general uncertainty surrounding experimental sciences, but because the conception is incomplete due to factual uncertainty that undermines the specificity of the inventor's idea of the invention. *Burroughs Wellcome Co.* v. *Barr Laboratories Inc.*, 40 F.3d 1223, 1229, 32 USPQ2d 1915, 1920 (Fed. Cir. 1994). Reduction to practice in effect provides the only evidence to corroborate conception (and therefore possession) of the invention. *Id.*

[54] *See Eli Lilly*, 119 F.3d at 1568, 43 USPQ2d at 1406.

[55] *See, e.g., Rasmussen*, 650 F.2d at 1214, 211 USPQ at 326–27 (disclosure of a single method of adheringly applying one layer to another was sufficient to support a generic claim to "adheringly applying" because one skilled in the art reading the specification would understand that it is unimportant how the layers are adhered, so long as they are adhered); *In re Herschler*, 591 F.2d 693, 697, 200 USPQ 711, 714 (CCPA 1979) (disclosure of corticosteriod in DMSO sufficient to support claims drawn to a method of using a mixture of a "physiologically active steroid" and DMSO because "use of known chemical compounds in a manner auxiliary

to the invention must have a corresponding written description only so specific as to lead one having ordinary skill in the art to that class of compounds. Occasionally, a functional recitation of those known compounds in the specification may be sufficient as that description."); *In re Smythe*, 480 F.2d 1376, 1383, 178 USPQ 279, 285 (CCPA 1973) (the phrase "air or other gas which is inert to the liquid" was sufficient to support a claim to "inert fluid media" because the description of the properties and functions of the air or other gas segmentizing medium would suggest to a person skilled in the art that appellant's invention includes the use of "inert fluid" broadly.). However, in *Tronzo* v. *Biomet*, 156 F.3d at 1159, 47 USPQ2d at1833 (Fed. Cir. 1998), the disclosure of a species in the parent application did not suffice to provide written description support for the genus in the child application.

[56] *See, e.g., Eli Lilly.*

[57] For example, in the molecular biology arts, if an applicant disclosed an amino acid sequence, it would be unnecessary to provide an explicit disclosure of nucleic acid sequences that encoded the amino acid sequence. Since the genetic code is widely known, a disclosure of an amino acid sequence would provide sufficient information such that one would accept that an applicant was in possession of the full genus of nucleic acids encoding a given amino acid sequence, but not necessarily any particular species. *Cf. In re Bell*, 991 F.2d 781, 785, 26 USPQ2d 1529, 1532 (Fed. Cir. 1993) and *In re Baird*, 16 F.3d 380, 382, 29 USPQ2d 1550, 1552 (Fed. Cir. 1994).

[58] *See Wertheim*, 541 F.2d at 263, 191 USPQ at 97 ("[T]he PTO has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in the disclosure a description of the invention defined by the claims.").

[59] *See* MPEP §§ 714.02 and 2163.06 ("Applicant should * * * specifically point out the support for any amendments made to the disclosure.").

[60] *See, e.g., In re Wright*, 866 F.2d 422, 425, 9 USPQ2d 1649, 1651 (Fed. Cir. 1989) (Original specification for method of forming images using photosensitive microcapsules which describes removal of microcapsules from surface and warns that capsules not be disturbed prior to formation of image, unequivocally teaches absence of permanently fixed microcapsules and supports amended language of claims requiring that microcapsules be "not permanently fixed" to underlying surface, and therefore meets description requirement of 35 U.S.C. 112.).

[61] *See, e.g., In re Robins*, 429 F.2d 452, 456–57, 166 USPQ 552, 555 (CCPA 1970) ("[W]here no explicit description of a generic invention is to be found in the specification * * * mention of representative compounds may provide an implicit description upon which to base generic claim language."); *In re Smith*, 458 F.2d 1389, 1395, 173 USPQ 679, 683 (CCPA 1972) (a subgenus is not necessarily implicitly described by a genus encompassing it and a species upon which it reads).

[62] *See, e.g., In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950–51 (Fed. Cir. 1999) ("To establish inherency, the extrinsic evidence "must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.'") (citations omitted).

[63] When an explicit limitation in a claim "is not present in the written description whose benefit is sought it must be shown that a person of ordinary skill would have understood, at the time the patent application was filed, that the description requires that limitation." *Hyatt* v. *Boone*, 146 F.3d 1348, 1353, 47 USPQ2d 1128, 1131 (Fed. Cir. 1998).

[64] *See, e.g., Johnson Worldwide Associates Inc.* v. *Zebco Corp.*, 175 F.3d at 993, 50 USPQ2d at 1613; *Gentry Gallery, Inc.* v. *Berkline Corp.*, 134 F.3d at 1479, 45 USPQ2d at 1503; *Tronzo* v. *Biomet*, 156 F.3d at 1159, 47 USPQ2d at 1833.

[65] *See, e.g., In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971).

[66] *Wertheim*, 541 F.2d at 263, 191 USPQ at 97.

[67] *See Rasmussen*, 650 F.2d at 1214, 211 USPQ at 326.

[68] *See In re Alton*, 76 F.3d 1168, 1176, 37 USPQ2d 1578, 1584 (Fed. Cir. 1996).

[FR Doc. 01–323 Filed 1–4–01; 8:45 am]
BILLING CODE 3510–16–U

CORPORATION FOR NATIONAL AND COMMUNITY SERVICE

Revision of Currently Approved Information Collection; Comment Request

AGENCY: Corporation for National and Community Service

ACTION: Notice.

SUMMARY: The Corporation for National and Community Service (hereinafter "Corporation"), as part of its continuing effort to reduce paperwork and respondent burden, conducts a preclearance consultation program to provide the general public and Federal agencies with an opportunity to comment on proposed and/or continuing collections of information in accordance with the Paperwork Reduction Act of 1995 (PRA95) (44 U.S.C. 3506(c)(2)(A)). This program helps to ensure that requested data can be provided in the desired format, reporting burden (time and financial resources) is minimized, collection instruments are clearly understood, and the impact of collection requirement on respondents can be properly assessed.

Currently, the Corporation is soliciting comments concerning the proposed revision of its Voucher and Payment Request Form (OMB #3045–0014).

Copies of the forms can be obtained by contacting the office listed below in the address section of this notice.

DATES: Written comments must be submitted to the office listed in the **ADDRESSES** section by March 6, 2001.

ADDRESSES: Send comments to Levon Buller, National Service Trust, Corporation for National and Community Service, 1201 New York Ave., NW., Washington, DC 20525.

FOR FURTHER INFORMATION CONTACT: Levon Buller, (202) 606–5000, ext. 383.

SUPPLEMENTARY INFORMATION: The Corporation is particularly interested in comments which:

- Evaluate whether the proposed collection of information is necessary for the proper performance of the functions of the Corporation, including whether the information will have practical utility;
- Evaluate the accuracy of the agency's estimate of the burden of the proposed collection of information, including the validity of the methodology and assumptions used;
- Enhance the quality, utility and clarity of the information to be collected; and
- Minimize the burden of the collection of information on those who are to respond, including through the use of appropriate automated, electronic, mechanical, or other technological collection techniques or other forms of information technology, *e.g.*, permitting electronic submissions of responses.

Background

The Corporation supports programs that provide opportunities for individuals who want to become involved in national service. The service opportunities cover a wide range of activities over varying periods of time. Upon successfully completing an agreed-upon term of service in an approved AmeriCorps program, a national service participant—an AmeriCorps member—receives an "education award". This award is an amount of money set aside in the member's name in the National Service Trust Fund. This education award can be used to make payments towards qualified student loan or pay for educational expenses at qualified post-secondary institutions and approved school-to-work opportunities programs. Members have seven years in which to draw against any unused balance.

The National Service Trust is the office within the Corporation that administers the education award